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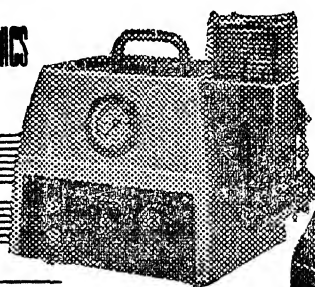
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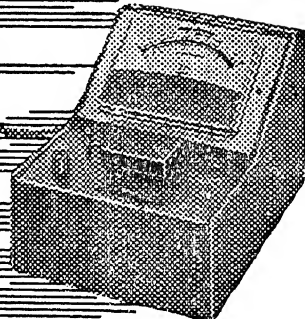
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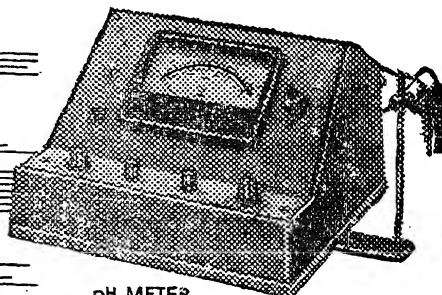
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# COEXISTING AEGIRINE AND MAGNESIORIEBECKITE FROM BABABUDAN HILLS MYSORE STATE

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**T**HE occurrence of magnesioriebeckite (Bababudanite) in a few horizons of the banded iron formations of the Bababudan Hills has been reported by earlier workers<sup>1-3</sup>. The coexistence of aegirine along with magnesioriebeckite has now been found in one of the horizons at the base of the scarp between Kavikalgandi and Manikyadhara falls. These silicate minerals form distinct thin bands associated with magnetite and quartz bands of the iron formation. The chemistry and the physical properties of the aegirine have been studied and are reported in this communication.

## MINERALOGY

Aegirine occurs as a semiradial grass-green prismatic aggregate (2-4 cm long), growing from a few points along the bedding plane. It is feebly pleochroic and the lamellar twinning is conspicuous. Cleavage is perfect on [110] and the basal pyroxene cleavages are distinct. No zoning in one and the same crystal could be observed. Aegirine is found to be optically negative,  $\alpha : z = 8^\circ$  (max) and the refractive indices measured are  $\alpha = 1.765$ ,  $\gamma = 1.829$ . The textural relationships suggest that aegirine has grown from magnesioriebeckite. A number of crystals of aegirine are also seen growing across the magnesioriebeckite bands (Fig. 1), suggesting its later formation. In contrast to

aegirine, the magnesioriebeckite forms distinct bands of slender acicular aggregates oriented parallel to each other. The colour of the latter is dark blue and has a pleochroic scheme: prussian blue to yellowish green to indigo and has a lower refractive index than that of aegirine.

Both aegirine and magnesioriebeckite were separated for chemical analysis. While the aegirine could be hand-picked, the isolation of magnesioriebeckite posed some problem, since the iron ore grains were found embedded in the mineral. The crushed sample is gravity separated with bromoform and the heavier fraction is boiled with concentrated hydrochloric acid. The insoluble fraction has been taken for chemical analysis. The analytical data are presented in Table I, along with the number of atoms per formula unit. The composition with more than 90 mole per cent of  $\text{NaFe}^{+3}\text{Si}_2\text{O}_6$  and low  $\text{H}_2\text{O}^+$  content clearly indicates that the mineral is aegirine. Besides, the percentage of  $\text{Na}_2\text{O}$  is higher than that of any of the known sodic amphiboles. The analysis shows that many of the major constituents have similar distribution in both the coexisting minerals. However, the amount of  $\text{MgO}$  and  $\text{H}_2\text{O}^+$  are undoubtedly partitioned in favour of the amphibole phase. The oxidation ratio,  $(\text{Fe}^{+3}/\text{Fe}^{+3} + \text{Fe}^{+2})$  in both the cases are high (0.94 and 0.81 for aegirine and magnesioriebeckite respectively). When the value of  $100 \text{ Fe}^{+2} : (\text{Fe}^{+2} + \text{Mg} + \text{Mn})$  vs.  $100 \text{ Fe}^{+3} : (\text{Fe}^{+3} + \text{Al}^{+6} + \text{Ti})$  is plotted for the amphibole, the point lies within the area of magnesioriebeckite. This excludes the possibility that the coexisting amphibole may be a crossite.

In order to confirm that the grass-green prismatic needles are aegirine, the X-ray powder diffraction pattern and also the infrared spectrum were taken. The observed reflections in the X-ray pattern could be indexed in the monoclinic system (Table II). The observed cell parameters are comparable to the reported values. Both  $a$ - and  $b$ -axes are shorter and the  $\beta$ -angle is also lower.

Figure 2 shows the infrared spectra of both aegirine and magnesioriebeckite. The difference in splitting and the number of distinguishable maxima in the silicate bands are very clear. In the region of  $600\text{--}800 \text{ cm}^{-1}$ , the characteristic peaks occur for



FIG. 1. Aegirine crystals growing across the magnesioriebeckite fibres. Black particles are magnetite,  $\times 40$ .

TABLE I  
Chemical composition of aegirine coexisting magnesioriebeckite

Aegirine				Magnesioriebeckite			
Wt. %		No. of ions Basis 6 (O) atoms		Wt. %		No. of ions Basis 24 (O) atoms	
SiO <sub>2</sub>	52.55	Si	2.017	SiO <sub>2</sub>	50.10	Si	7.394
TiO <sub>2</sub>	0.00	Al <sup>4</sup>	0.000	TiO <sub>2</sub>	0.00	Al <sup>4</sup>	0.563
		2.017				7.957	
Al <sub>2</sub> O <sub>3</sub>	0.26	Al <sup>6</sup>	0.012	Al <sub>2</sub> O <sub>3</sub>	4.08	Al <sup>6</sup>	0.000
Fe <sub>2</sub> O <sub>3</sub>	31.47	Ti	0.000	Fe <sub>2</sub> O <sub>3</sub>	21.05	Ti	0.000
FeO	1.84	Fe <sup>+3</sup>	0.911	FeO	4.60	Fe <sup>+3</sup>	2.428
MnO	0.02	Mg	0.023	MnO	0.01	Mg	2.217
MgO	0.40	Fe <sup>+2</sup>	0.059	MgO	10.08	Fe <sup>+2</sup>	0.568
CaO	0.84	Mn	0.001	CaO	0.14	Mn	0.001
		1.006				5.214	
Na <sub>2</sub> O	12.10	Na	0.902	Na <sub>2</sub> O	6.12	Na	1.752
K <sub>2</sub> O	0.17	Ca	0.035	K <sub>2</sub> O	0.74	K	0.139
H <sub>2</sub> O <sup>+</sup>	0.28	K	0.008	H <sub>2</sub> O <sup>+</sup>	2.03	Ca	0.022
		0.945				1.912	
H <sub>2</sub> O <sup>-</sup>	0.19			H <sub>2</sub> O <sup>-</sup>	0.23	OH	1.974
							1.974
	100.12				99.98		

TABLE II

X-ray powder diffraction data of aegirine

Cr K $\alpha$  radiation 114.6 mm camera.  $a = 9.653 \text{ \AA}$ ,  
 $b = 8.655 \text{ \AA}$ ,  $c = 5.267 \text{ \AA}$ ,  $\beta = 105^\circ 54'$ .  $Z = 4$ , Space  
group  $C_{2h}^2$ ,  $D = 3.530$ .

$d(\text{\AA})$	$hkl$	Relative intensity	$d(\text{\AA})$	$hkl$	Relative intensity
6.328	110	25	1.583	440	20
4.332	020	20	1.560	113	5
3.290	021	30	1.526	610	15
3.042	201	5	1.463	203	10
2.948	221	100	1.398	151	10
2.865	211	100	1.390	450	70
2.328	002	60	1.365	630	10
2.435	012	60	1.326	700	25
2.169	040	8	1.289	114	25
2.100	140	45	1.266	004	25
2.006	202	15	1.254	014	5
1.921	401	8	1.243	442	3
1.858	500	5	1.237	070	5
1.808	430	5	1.227	392	20
1.714	213	25	1.215	024	8
1.648	042	3	1.203	413	10
1.626	250	5	1.160	800	3
1.606	601	35	..	..	..

the glaucophane-riebeckite series. These are distinctly different in the case of aegirine, with the absence of bands around 790, 695 and 670  $\text{cm}^{-1}$  (Fig. 2—II).

#### PETROGENETIC SIGNIFICANCE

The paragenesis of aegirine-riebeckite pair covers a wide range, such as alkali, igneous rocks, carbonates, glaucophinitic schists, green schists, meta-

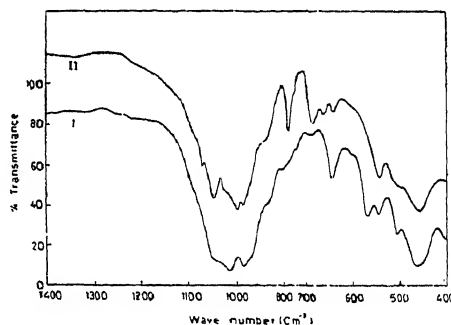


Fig. 2. Infrared spectra of I. Aegirine and II. Magnesioriebeckite.

cherts and alkalimetamorphic environments. Aegirine and riebeckite found in iron formations are explained to be due to the reaction of soda-rich water on haematite cherts<sup>4</sup>, by diagenesis<sup>5</sup>, sodametasomatism<sup>6</sup>. The soda for the formation of magnesioriebeckite in sediments of Bababudan Hills was thought to have been derived from the surrounding rocks of spilitic affinities<sup>6,11</sup>. Later the occurrence of acmite at the contact of intrusive quartz-albite-dolerites in the ferruginous quartzites of Kalhatti was attributed to sodametasomatism<sup>12</sup>. Since the magnesioriebeckite-aegirine zones occur as distinct bands and are not spatially related to basic sills, soda metasomatism need not be a factor in their genesis. The experimentally determined upper stability field of magnesioriebeckite in a silica deficient system by Ernst<sup>8</sup> show that at low vapour pressure (less than 200 atm.) and temperature

range of 800–950° C, the conversion to haematite, magnesioferrite, olivine, aegirine and vapour takes place. Above this pressure, magnesioriebeckite melts incongruently. Similarly, riebeckite breaks down around 750° C and below 1,500 atm. water vapour pressure to aegirine, fayalite, magnetite, quartz and vapour. The coexistence of magnesioriebeckite and aegirine can be explained on these lines; the vapour pressure built up in such a case may be due to the partial pressure of oxygen in the oxide facies rocks and carbon dioxide in carbonate facies rocks<sup>9</sup>. The formation of magnesioriebeckite, in turn, may be explained by the concept of Cilliers and Genis<sup>10</sup>; accordingly riebeckite is formed by lithification and diagenesis or under greenschist facies metamorphism of attapulgite-rich clay admixtures containing precipitates of iron and silica along with alkali solutions.

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## AN OCCURRENCE OF GADOLINITE NEAR KARATTUPPATTI, MADURAI DISTRICT, TAMIL NADU

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### ABSTRACT

Chemical and spectrographic analyses of a greenish-black radioactive mineral from a zoned pegmatite near Karattuppatti showed 51.84%  $\text{Ce}_2\text{O}_3 + \text{La}_2\text{O}_3 + \text{Y}_2\text{O}_3$ , 9.0%  $\text{BeO}$ , 0.54%  $\text{U}_3\text{O}_8$ , 15.7%  $\text{Fe}_2\text{O}_3 + \text{FeO}$ , 22.04%  $\text{SiO}_2$ , and minor amounts of Er, Gd, Yb, Dy, Ho, Lu, Tm, B, Cr, Mg, Mn, and Zr. X-ray diffractometry confirmed it to be gadolinite—the second of its type to be reported from India. The presence of cerium as a major element in the mineral establishes it to be the variety cergadolinite.

### INTRODUCTION

**A**N *in situ* boulder weighing about 15 kg and consisting of two distinct minerals—a golden yellow beryl and a dark greenish-black mineral—was located in a zoned pegmatite about a km southwest of Karattuppatti village (10° 03'–77° 55') (Survey of India Toposheet No. 58 F/16) in Tirumangalam Taluk, Madurai District. X-ray diffractometric, chemical and spectrographic analyses of the greenish-black mineral have established it to be gadolinite—an uncommon rare-earth mineral ( $\text{Be}_2\text{FeY}_2\text{Si}_2\text{O}_{10}$ ), the occurrence of which has not been recorded since Holland's report<sup>1</sup> on gadolinite from Hosainpura (Banaskanta District, Gujarat). This paper presents a description of the geological setting, mineralogical and geochemical features of the Karattuppatti gadolinite occurrence.

### GEOLOGICAL SETTING

The area around Karattuppatti (Fig. 1) exposes Precambrian migmatitic gneiss composed of garnet, biotite, hornblende, magnetite, feldspar and quartz in varying proportions. This gneiss is flanked on the north by highly feldspathic garnetiferous

gneiss, and in the south by quartzite interbanded with the migmatitic gneiss. Distinct bands of calc- and pyroxene-granulite, too small to be represented separately on the map, occur interbanded with the gneissic rocks. Zoned discordant pegmatites carrying rare-earth minerals and beryl are confined to the gneissic rocks and are absent in the quartzite. Foliation in the rock formations trends about east-west with sub-vertical to steep southerly dips, generally conforming to the broad regional trends of the formations.

### MINERALOGICAL ASPECTS

The gadolinite-bearing pegmatite has a central zone of perthite surrounded by a perthite-quartz-plagioclase zone and a peripheral zone of quartz-feldspar-muscovite pegmatite (Fig. 1). Assemblages of radioactive rare-earth minerals consisting of allanite, polycrase and gadolinite, as also golden yellow, blue and green beryl, tourmaline and muscovite are associated with the two outer pegmatite zones. The rare-earth minerals and beryl occur as disseminations, small segregations and pods in the pegmatite, and appear as float in the debris around often ranging upto 15 cm in length,

In hand specimen, the gadolinite is greenish-black and opaque, and in thin sections, is bottle green. It is radioactive and has a conchoidal

fracture. With the increasing use of rare-earths and beryllium in the television, electronic and aerospace industries, and in nuclear reactors, it

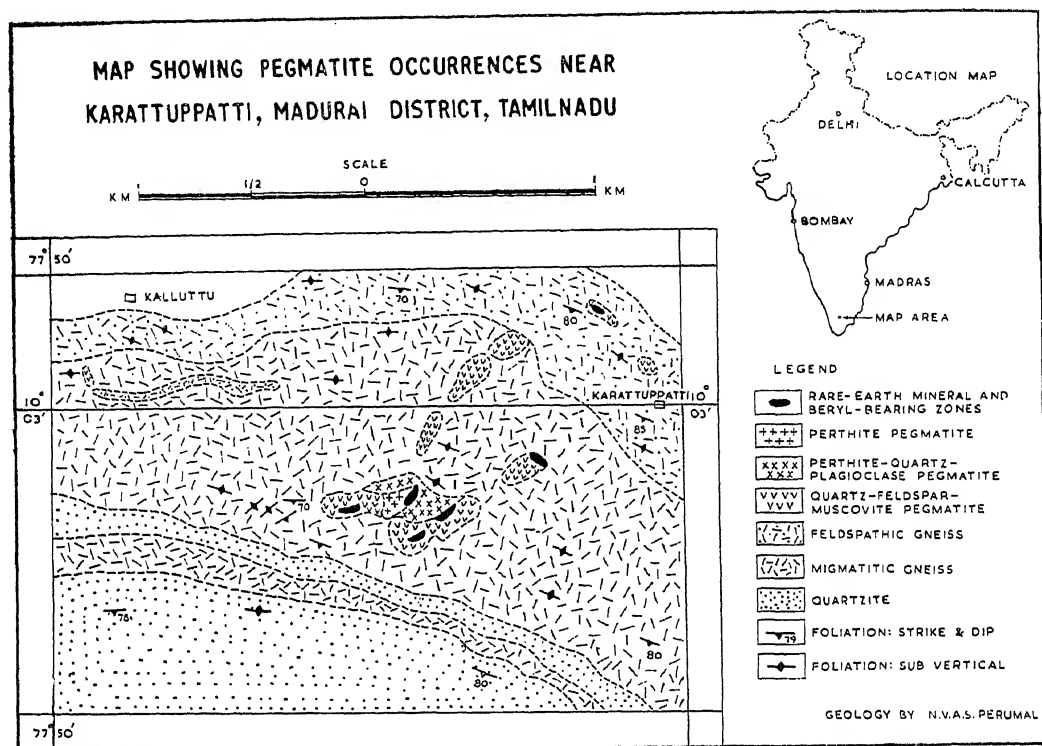


FIG. 1

fracture, vitreous lustre, hardness of 7, and a Specific Gravity of 4.167.

#### GEOCHEMICAL FEATURES

The mineral has 51.84% of combined cerium, lanthanum and yttrium earths, 22.04%  $\text{SiO}_2$ , 15.7% of total iron oxides, and 9.0%  $\text{BeO}$ . These data compare very well with the theoretical composition of gadolinite<sup>2</sup>—( $\text{Y}_2\text{FeBe}_3\text{Si}_2\text{O}_{10}$ )—(55.40% rare-earths, 22.20%  $\text{SiO}_2$ , 13.2%  $\text{FeO}$  and 9.2%  $\text{BeO}$ ) and also with the compositions of gadolinites reported from the U.S.A., U.S.S.R., and Sweden (Table I). The presence of 0.54%  $\text{U}_3\text{O}_8$  in the Karattuppatti gadolinite sample is responsible for its observed metamictization. Spectrographic analysis revealed also the presence of Er, Gd, Yb, Dy, Ho, Lu, Tm, B, Cr, Mg, Mn, and Zr in the sample.

#### CONCLUSION

The presence of cerium as a major element in the Karattuppatti gadolinite establishes it to be the variety cergadolinite. The occurrence described here is only the second of its kind to be reported

TABLE I  
Chemical analyses (%)

	1	2	3	4
$\text{Ce}_2\text{O}_3$	51.84	32.33	15.44	6.52
$\Sigma \text{La}_2\text{O}_3$				
$\Sigma \text{Y}_2\text{O}_3$		22.24	36.92	39.27
$\text{U}_3\text{O}_8$	0.54	0.89	..	..
$\text{ThO}_2$	..	0.46	..	..
$\text{SiO}_2$	22.04	22.13	24.84	25.16
$\text{Fe}_2\text{O}_3$	15.7	1.13	..	2.15
$\text{FeO}$		10.43	9.67	12.40
$\text{BeO}$	9.0	7.19	8.82	9.37
$\text{Al}_2\text{O}_3$	..	2.34	1.27	..
$(\text{Zr}, \text{Ti})\text{O}_2$	..	..	1.58	..
$\text{CaO}$	..	0.34	..	1.11
$\text{MgO}$	..	0.14	..	..
$\text{Na}_2\text{O}$	..	..	0.64	..
$\text{B}_2\text{O}_3$	..	0.86	..	2.32
$\text{H}_2\text{O}$	..	..	0.74	1.28

- Gadolinite from Karattuppatti, Madurai District, India.
- Cergadolinite from Colorado, U.S.A.<sup>2</sup>
- Cergadolinite from Tuva, U.S.S.R.<sup>2</sup>
- Gadolinite from Ytterby, Sweden.<sup>2</sup>

would be worthwhile to systematically investigate the rare mineral potential of small as well as large complex pegmatites of India.

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## HETEROSIS IN CHROMOSOME BEHAVIOUR OF EGG-PLANT

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AND

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#### ABSTRACT

Chiasma frequency in 6 varieties of egg-plant (*Solanum melongena* L.) and their 10 hybrids, 5 highest yielding and 5 lowest yielding, out of possible 30 has been studied. Varieties and hybrids differed from each other with regard to mean chiasma frequency. The chiasma frequency is reported to be genetic and probably under the control of polygenes. Four hybrids showed heterosis in chiasma frequency over their better parent, the maximum being 30.8%.

#### INTRODUCTION

THE study of heterosis in chromosome behaviour and the genetics of chiasma frequency is a new line of work in the history of cytogenetics. Consequently, not much work has been done on this aspect. In 1936 Lamm's work on rye for the first time showed the effects of inbreeding on chromosome behaviour at meiosis where the inbred lines showed a reduction in chiasma frequency which, in many cases, was accompanied by considerable asynapsis<sup>1</sup>. Inbred lines varied from one to the other just as would be expected from a segregation of genes controlling chiasma formation. Associated with differences in chiasma frequency was a difference in the size of bivalents between some lines. This was comparable to the genotypically controlled variation in chromosome size reported in *Lolium* by Thomas<sup>2</sup>. Thereafter, a few more studies on the behaviour of chromosome of rye were reported in detail<sup>3-7</sup>. The present paper is first of its kind on egg-plant.

#### MATERIALS AND METHODS

Six egg-plant varieties-purple slender, green long, Type 4, Purple Round, 9LO and K. 6312- and their 5 highest yielding and 5 lowest yielding crosses out of a 6 × 6 diallel set constituted the material for the present investigation. While chiasma frequency in parents was scored in flower buds

collected in the month of June 1967 and 1968, in the hybrids the same was done in buds collected only in June, 1968. Flower buds of suitable size were fixed in 1:3 acetoalcohol for 24 hours and thereafter transferred to 70% alcohol. Meiotic slides were prepared in 1.5% propiono-carmin by the usual method of Darlington and La Cour<sup>1</sup>. Chiasma frequencies were scored at diakinesis and expressed in terms of number of chiasmata per cell and per bivalent.

#### RESULTS AND DISCUSSION

Chiasma frequency as observed in 6 varieties during 1967 and 1968 are given in Table I. Chiasma frequency of the hybrids and heterosis in them in regard to this character are presented in Table II.

It appears from Table I that varietal differences with regard to chiasma frequency during 1967 and 1968 are not much. Similarly the hybrids (Table II) excepting one also do not differ much from each other. But the distribution of chiasma frequency in parents as well as in hybrids, however, appears to be continuous. Thus it is probably under the control of polygenes like that reported in rye by Rees<sup>3-5</sup>. This observation is supported by the fact that the amount of heterosis with regard to this character is different in different hybrids as one expects in case of a polygenic trait,

TABLE I

*Chiasma frequency per cell and per bivalent in different varieties. The latter is given in parenthesis*

Varieties with abbreviations	Chiasma frequency	
	June, 1967	June, 1968
Purple Slender (PS)	21.60 (1.77)	21.02 (1.75)
Green Long (GL)	22.36 (1.87)	20.35 (1.70)
Type 4 (TF)	24.83 (2.07)	21.08 (1.76)
Purple Round (PR)	21.87 (1.82)	21.91 (1.83)
9LO (NL)	22.62 (1.89)	22.32 (1.86)
K. 6312 (KS)	22.65 (1.89)	20.39 (1.70)
Mean	22.60 (1.88)	21.18 (1.77)

TABLE II

*Chiasma frequency in hybrids and heterosis in them*

Hybrids	Chiasma frequency per cell (per bivalent)	Heterosis in % over	
		Mid-parent	Better parent
GL × PS	21.78 (1.82)	5.32	3.63
NL × PS	21.42 (1.79)	-1.16	-4.05
NL × GL	29.20 (2.43)	36.86	30.80
PR × TF	21.86 (1.82)	1.68	-0.24
KS × GL	21.24 (1.77)	4.28	4.15
TF × KS	21.39 (1.78)	3.12	1.45
PS × TF	20.98 (1.75)	-0.35	-0.49
GL × PR	21.48 (1.79)	1.67	-1.96
PR × KS	21.06 (1.76)	-0.43	-3.88
NL × KS	21.44 (1.79)	0.38	-3.96
Mean	22.18 (1.85)	5.15	2.58

The magnitude of difference in chiasma frequency observed in 1967 and 1968 in each variety is not similar (Table I). Purple Slender, Purple Round and 9LO are more stable while Green Long, Type 4 and K. 6312 are prone to the seasonal variations. Similar result in rye has been reported<sup>5</sup>.

The chiasma frequency in Purple Slender × Type 4 which is 20.98 is lesser than that of either of the parents; it shows that genes for low chiasma frequency in this cross show overdominance while in Purple Round × K. 6312 and 9LO × Purple Slender, the chiasma frequency being between poor parent and mid-parent, dominance of these genes is partial only. On the other hand in Green Long × Purple Slender, 9LO × Green Long, K. 6312 × Green Long and Type 4 × K. 6312 genes for higher chiasma frequency show overdominance. However, the dominance of genes responsible for higher chiasma frequency in Purple Round × Type 4, Green Long × Purple Round and 9LO × K. 6312 is partial only because the Chiasma frequency in these crosses is between mid-parent and superior parent value. The heterosis in chiasma frequency positive or negative, might also be due to inter-allelic interaction. To establish exact nature of the genes controlling chiasma frequency in egg-plant, a more detailed study like that of diallel cross study of chiasma frequency as has been done in rye<sup>7</sup> is required.

Cross 9LO × Green Long showing the highest heterosis of 30.8% over better parent and also giving the highest yield is expected to produce largest number of segregants with various character combinations in  $F_2$ . Therefore, there is maximum possibility of getting a desired recombinant in  $F_2$  of this cross.

#### ACKNOWLEDGEMENTS

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## LETTERS TO THE EDITOR

### INTRAMOLECULAR FORCE FIELDS AND MEAN AMPLITUDES OF VIBRATION OF $\text{IO}_6^{5-}$ ION

VERY little is known about the existence and spectroscopic constants of octahedral oxy-compounds of halogens. Recently, Siebert and Wieghardt<sup>1</sup> have reported the infrared and Raman spectra of octahedral  $\text{IO}_6^{5-}$  anion in the salts  $\text{Li}_5\text{IO}_6$ ,  $\text{Ba}_5(\text{IO}_6)_2$  and  $\text{NaBa}_2\text{IO}_6$ . The assigned vibrational frequencies in  $\text{Li}_5\text{IO}_6$  for the ion  $\text{IO}_6^{5-}$  (in  $\text{cm}^{-1}$ ) are  $\nu_1(a_1) = 656$ ,  $\nu_2(e) = 641$ ,  $\nu_3(f_{1u}) = 691$ ,  $\nu_4(f_{1u}) = 444$ ,  $\nu_5(f_{2g}) = 470$ ,  $\nu_6(f_{2u}) = 332.3$ . Using the available molecular structural and vibrational data, we report the molecular vibration analysis of the ion in question. Three types of vibrational potential functions were used, viz., modified orbital valency force field (MOVFF), modified Urey-Bradley force field (MUBFF) and general quadratic potential

TABLE II			
Mean amplitudes of vibration (in Å)			
Distances	T=0° K	T=298.16° K	T=500° K
I—O bonded	0.0421	0.0438	0.0487
O...O non-bonded linear	0.0571	0.0597	0.0668
O...O non-bonded non-linear	0.0702	0.0774	0.0899

tion for bonded (I—O) distance in the series  $\text{IO}_6^{5-}$ ,  $\text{IO}_4^-$  and  $\text{IO}_3^-$  are respectively 0.042 Å, 0.039 Å and 0.039 Å and the corresponding force constants are 3.818, 6.198 and 5.292 mdyne/Å. This shows that the relative bond strength of iodine-oxygen bond is in the order  $\text{IO}_6^{5-} < \text{IO}_4^- < \text{IO}_3^-$  and the mean amplitudes are highly characteristic.

TABLE I  
Intramolecular force field constants (mdyne/Å)

MOVFF	MUBFF	GQPF	$F_{33}$	$F_{34}$	$F_{44}$	$\xi_{33}$	$\xi_{44}$
K = 2.621	K = 3.601	$f_r = 3.554$	3.854 <sup>a</sup>	0.637 <sup>a</sup>	0.723 <sup>a</sup>	0.201	0.299
D = 1.182	H = 0.490	$f_{rr} = 0.174$	3.467 <sup>b</sup>	0.050 <sup>b</sup>	0.687 <sup>b</sup>		
F = 0.349	F = 0.056	$f'_{rr} = 0.607$	3.702 <sup>c</sup>	0.267 <sup>c</sup>	0.662 <sup>c</sup>		
F' = 0.288	k = 0.234	$f_{ra} - f'_{ra} = 0.125$	3.855 <sup>d</sup>	0.646 <sup>d</sup>	0.726 <sup>d</sup>		
k = 0.040	h = 0.083	$f_a - f'''_{aa} = 0.526$	3.594 <sup>e</sup>	0.133 <sup>e</sup>	0.662 <sup>e</sup>		
$\frac{h}{2} = 0.194$		$f_{aa} - f''_{aa} = 0.047$					
		$f'_{aa} - f_{aa}''' = 0.047$					
a — MOVFF,	b — MUBFF,	c — $L_{12} = \text{O}$ ,	d — $L_{21} = \text{O}$ ,	e = PED.			

function (GQPF) as adopted by Ramaswamy<sup>2</sup>, So<sup>3</sup> and Pistorious<sup>4</sup> respectively. The F—G matrix elements and symmetry coordinates<sup>4</sup> were those used in our earlier studies<sup>5</sup>. The potential constants (Table I) reproduce the observed frequencies with appreciable accuracy. Spectroscopic mean amplitudes of vibration for bonded iodine oxygen and non-bonded oxygen-oxygen distances have also been computed (Table II) following the method of Cyvin<sup>6</sup>. The second order eigenvalue problem was examined by several approximate methods (Table I) and the coriolis coupling constants are reported.

It is interesting to compare the molecular constants of  $\text{IO}_6^{5-}$  anions with those of its lower molecular species. The mean amplitudes of vibra-

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# URANIUM CONTENT IN PLANTS— A PRELIMINARY REPORT

URANIUM is a constituent of living matter but the biological significance of the apparently universal distribution of uranium in organisms is not well understood. Uranium has been reported as a normal component of protoplasm<sup>1</sup>. Limited information on uranium content in organisms shows that its concentration varies between  $10^{-4}\%$  and  $10^{-9}\%$  by weight<sup>2,3</sup>. Formation of some uranium deposits have been attributed to fixation of uranium by some algae<sup>4</sup> and similarly there is a suggestion that micro-organisms may also have played a role in the concentration of the uranium and vanadium from dilute solutions<sup>5</sup>.

The present investigation was undertaken to work out the uranium content in plants, growing in different habitats at Kurukshetra with a view to correlate the uranium content to the plant habitat.

TABLE I

*Uranium content in various plant species*

Ecological group	Name of the plant	Average uranium concentration (ppm)
Hydrophyte	1. <i>Potamogeton crispus</i>	13.7
	2. <i>Vallisneria spiralis</i>	26.7
	3. <i>Rhizoclonium</i> sp.	3.6
Mesophyte	4. <i>Dahlia</i> sp.	3.0
	5. <i>Allium cepa</i>	1.4
	6. <i>Ageratum conyzoides</i>	2.6
	7. <i>Lathyrus odoratus</i>	1.6
	8. <i>Medicago sativa</i>	0.8
Xerophyte	9. <i>Bryophyllum</i> sp.	1.2
	10. <i>Opuntia</i> sp.	0.9
	11. <i>Calatropis procera</i>	1.5
	12. <i>Agremone mexicana</i>	0.8
	13. <i>Alhagi camelorum</i>	2.4
	14. <i>Sorghum halepense</i>	1.2
	15. <i>Cycas circinalis</i>	3.5
	16. <i>Biota indica</i>	1.6

Sixteen plant species (Table I) from different ecological habitats, as per Daubenmire's<sup>6</sup> classification, collected from Kurukshetra, were analyzed for uranium content by fission track technique. Small portions of plant material, obtained by mixing different parts, were dried in an oven at  $150^{\circ}\text{C}$  for 24 hours and thereafter fused in contamination-free silica crucibles in a furnace at  $700^{\circ}\text{C}$

for 2 hours. 50 mg as from each plant material was thoroughly mixed with 100 mg of methyl cellulose powder and pressed into a flat pellet of about 1.3 cm diameter. The pellets were covered with lexan plastics and were irradiated with  $10^{15}$  nvt. thermal neutron dose at CIRUS atomic Reactor, Trombay. Fission fragments from uranium in the samples impinge upon the covering plastic and leave discernible tracks. The resulting fission tracks were counted in the lexan plastic after chemical etching<sup>7</sup> with sodium hydroxide solution. The uranium content was determined by comparing the number of such tracks to the number of tracks induced in a plastic which covered a similar pellet of standard glass irradiated simultaneously. Analytical error determined by statistical track counts in the samples and in co-irradiated standards comes to  $\leq \pm \%$  for the data reported here.

Uranium content in various species varies from 0.8 ppm to 26.7 ppm (Table I). Hydrophytes were found to have maximum internal borne uranium and this could be due to their capacity of fixing uranium in water as shown by algae and some micro-organisms<sup>4,5</sup>.

There is no appreciable difference in uranium content between mesophytes and xerophytes but the three succulent xerophytes (*Bryophyllum*, *Opuntia* and *Calatropis*) have almost equal uranium content. As the mesophytes and xerophytes, used for the present study, were collected from within a radius of only 5 km, the variation in habitat may be negligible and hence the uranium content in the two classes is not appreciably different.

Radiation from internally borne uranium could be a possible cause of spontaneous mutations, whose origin is much debated<sup>8</sup>. It may be speculated that these internally borne radiations are insufficient to cause mutations. But the studies of Wallace and Dobzhansky<sup>9</sup> indicate that even small amounts of radiation are able to induce a calculable number of mutations. Therefore, it would be useful to work out the actual dose of radiation produced by the uranium present to decide conclusively whether its presence can partly or wholly account for spontaneous mutations in organisms.

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#### ANALYTICAL APPLICATION OF MALONDIANILIDE OXIME FOR ESTIMATION OF COPPER, NICKEL AND COBALT

SEVERAL oximes of orthohydroxy aldehydes and ketones were examined by Ephraim<sup>1</sup>, who found them to be selective reagents for copper in weakly acid medium. Fiegl and Bondi's<sup>2</sup> work on oximes of aliphatic compounds have shown that for grouping to be specific for copper, it must be a part of an aromatic structure as the presence of more acidic hydrogen such as that present in phenolic OH, is apparently necessary. It is possible that the difference in steric structure brought about by the aromatic binding may also be having some effect. Another prominent reagent of this class is resacetophenone oxime (Rapox) used by several workers<sup>3-6</sup>. Schiff's bases formed by *o*-hydroxy-aldehydes give identical results as their oximes<sup>7-9</sup>. In spite of the advantages due to the intense colouration of copper salt in the case of 1-acetyl-2-naphthol-oxime<sup>10</sup>, the efficacy of salicylaldoxime for the estimation of copper was shown by Ephraim (*loc. cit.*).

A careful search of the literature shows that oximes of substituted malonic group, though reported in the literature<sup>11</sup>, do not appear to have been investigated to find their usefulness. The present paper is one of the series of the work undertaken, (a) to see if malondianilide oxime (HINMA) is as useful and effective as other reagents for copper, (b) to study the reactions of similar oximes containing other substituents in the malonic group, particularly with transition metals and (c) to see if and under what conditions the oximes of the malonic group derivatives can be used for the estimation of copper, nickel and cobalt.

Malondianilide oxime (HINMA) gives a dull-brown and bright olive-green bulky precipitates with copper (II) ions in absolute alcohol and

60 : 40 aqueous alcoholic solutions respectively. Dull-brown precipitate of copper (II) complex is obtained when 0.125 M solution of HINMA is gradually added with constant stirring to a 0.125 M alcoholic solution of cupric chloride so that the ligand to metal ion ratio in the mixture was 2 : 1. The precipitate gets readily coagulated on slight warming on water bath. It is carefully filtered under suction, washed repeatedly with alcohol and finally with acetone and dried at 68°C. In the case of aqueous alcoholic solutions a bright flocculent olive-green precipitate is obtained when ligand to metal ratio of (2 : 1) in the mixture was maintained after slowly adding with constant stirring the solution of ligand to metal ion. The precipitate readily settles down. It is warmed on water bath for a while, cooled and filtered under suction. It is washed with 60 : 40 alcohol, finally by acetone and dried at 68°C. The process is quite rapid, quantitative and efficient.

On adding slowly with constant stirring alcoholic solution of the reagent (0.125 M, pH 3.1) to aqueous solution of copper chloride (0.125 M, pH 4.6), the pH of the solution decreases as the complex is formed and copper is completely precipitated at 2.2 pH when the proportion of metal to reagent in the solution was 1 : 2. It was not necessary to use any external reagent for maintaining the pH range 3.9 to 2.2 when the complex is formed.

Nickel (II) gives a light pink amorphous complex when to the aqueous solution of nickel chloride (0.1 M, 5.3 pH) alcoholic solution of the reagent (0.1 M) was added slowly with constant stirring so that the metal to reagent ratio in the mixture was 1 : 2. The precipitation range for nickel complex was found to be 5.1 to 2.85 pH.

On adding alcoholic solution of the reagent (0.1 M) to aqueous cobalt chloride solution (0.1 M, 5.7 pH) a bright red brown complex was formed in a pH range 5.2 to 2.9 when at 2.9 pH the metal to reagent ratio in the mixture was 1 : 3.

Tentatively it is observed that the complexes formed by nickel (II) and cobalt (III) were not quantitative.

In these preliminary tests it was noticed that the reagent gave selective precipitation of copper (II) from the aqueous mixtures of Cu(II)-Ni(II), Cu(II)-Co(III) and Cu(II)-Ni(II)-Co(III).

In the mixtures of the metal salt solution copper complex is preferentially precipitated first and only when the metal to reagent ratio exceeds 1 : 2 with reference to Cu(II) in the mixture distinct pale pink nickel complex slowly begins to separate. The formation of nickel complex is rather slow while

TABLE I

Metal salt solutions (aqueous) 0.1 M.		Reagent solution (alcoholic) 0.1 M.		
Metal ions in solution	pH of equi-molar mixture (10 ml)	Amount of reagent added (alcoholic) (ml.)	pH of the mixture	Result
Cu (II), Ni (II)	4.6	10	2.1	Only Cu (II) complex formed
Cu (II), Co (III)	4.7	10	2.1	"
Cu (II), Ni (II), Co (III)	4.9	10	2.2	"
(Glacial acetic acid)				
Cu (II), Ni (II)	4.6	10 or excess	0.7	Only Cu (II) complex formed
Cu (II), Co (III)	4.7	10 or excess	0.8	"
Cu (II), Ni (II), Co (III)	4.9	10 or excess	1.1	"

cobalt complex is formed appreciably slowly compared to copper and nickel complexes.

When the solution of reagent in glacial acetic acid was used only copper complex is formed. Presence of acetic acid inhibits the formation of nickel (II) and Co(II) complexes.

The results are summarised in Table I.

It is therefore considered that the reagent is selective to copper (II) and may prove very useful in quantitative separation and estimation of copper (II) from the mixtures of these metal salt solutions. Interference of the other ions and the structural study of these complexes is in progress.

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## CRYSTALLINE CONSTITUENTS OF EUPHORBACEAE

### Part XIV<sup>1</sup>. Isolation of Epitaraxerol from *Euphorbia royleana* Boiss.

In connection with our study on triterpene rearrangements, the stems of *Euphorbia royleana*<sup>2,3</sup> were extracted for glut-5-en-3 $\beta$ -ol. During our re-examination, friedelan-3 $\alpha$ -ol, lupeol,  $\beta$ -amyrin,  $\beta$ -sitosterol and a new triterpene were identified in the extract, besides the already reported triterpenes, namely, taraxerol<sup>2</sup> and glut-5-en-3 $\beta$ -ol<sup>3</sup>.

From the petroleum ether extract of the stems (5 Kg), fractional crystallisation furnished taraxerol, m.p. 280–1°, ( $\alpha$ )<sub>D</sub>  $\pm$  0° (C, 1.0 in CHCl<sub>3</sub>), ( $R_f$  value: 0.3; benzene; silica gel 'C'; yield: 5 g.), glut-5-en-3 $\beta$ -ol, m.p. 206–8°, ( $\alpha$ )<sub>D</sub> + 62° (C, 1.1 in CHCl<sub>3</sub>), (yield: 2 g) and a new triterpene, m.p. 259–60°, ( $\alpha$ )<sub>D</sub> + 2° (C, 1.0 in CHCl<sub>3</sub>), ( $R_f$  value: 0.35; benzene; silica gel 'C'; yield: 1 g).

The brown oily residue (30 g) was chromatographed on silica gel (450 g) and eluted with benzene and benzene: ethyl acetate (9:1). Fractional crystallisation of the eluate furnished friedelan-3 $\alpha$ -ol, m.p. 300–1°, ( $\alpha$ )<sub>D</sub> + 20° (0.2 g),  $\beta$ -amyrin, m.p. 199–200°, ( $\alpha$ )<sub>D</sub> + 94° (0.5 g), lupeol, m.p. 215–216°, ( $\alpha$ )<sub>D</sub> + 27° (0.5 g),  $\beta$ -sitosterol, m.p. 136–7° ( $\alpha$ )<sub>D</sub> – 37° (2 g), glut-5-en-3 $\beta$ -ol (1 g), taraxerol (20 g) and the new triterpene (0.5 g). These triterpenes were identified through their derivatives and by comparison with authentic samples (m.m.p. and I.R.).

The new triterpene crystallised from CHCl<sub>3</sub>: MeOH as shining needles, (C<sub>30</sub>H<sub>50</sub>O, T.M. test positive) and formed a mono acetate, colourless needles, m.p. 279–80°, ( $\alpha$ )<sub>D</sub> – 14° (C, 1.0 in CHCl<sub>3</sub>).

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N.M.R. 60 MHz in  $\text{CDCl}_3$ :  $\delta$  0.88–7.2 (m) 8 methyls,  $\delta$  2.08 (s) one acetoxy,  $\delta$  4.61–4.71 (m)  $\alpha$ -hydrogen on acetoxy and  $\delta$  5.46 (m) olefinic proton. Its benzoate crystallised from  $\text{CHCl}_3$ -MeOH as plates, m.p. 267–8°, ( $\alpha$ )<sub>D</sub> –1° (C, 1.0 in  $\text{CHCl}_3$ ).

Oxidation of the triterpene with chromium trioxide-pyridine complex furnished a ketone, shining plates, m.p. 240–2°, ( $\alpha$ )<sub>D</sub> +12° (C, 1.0 in  $\text{CHCl}_3$ ), identical with taraxerone (m.m.p. and I.R.). Therefore, the original alcohol may be an epimer of taraxerol.

The characteristic reaction of taraxerol is its rearrangement to  $\beta$ -amyrin with  $\text{HCl-HOAc}$ . Epitaraxerol should exhibit a similar re-arrangement and epi- $\beta$ -amyrin is expected. When the rearrangement was effected with epitaraxeryl acetate, epi- $\beta$ -amyrin acetate<sup>4</sup>, m.p. 166–8°, ( $\alpha$ )<sub>D</sub> +40°, was obtained and identified with an authentic sample (m.m.p. and I.R.).

Finally, conclusive evidence for the structure of epitaraxerol was obtained by its synthesis from taraxerone (400 mg) by Meerwein-Ponndorff reduction using aluminium isopropoxide (6 g) in isopropyl alcohol (10 ml). As expected, the product was a mixture of epimeric alcohols which were separated by fractional crystallisation from benzene. The first fraction was epitaraxerol, m.p. 259–60°, ( $\alpha$ )<sub>D</sub> +2° (C, 1.0 in  $\text{CHCl}_3$ ) (160 mg, 40%) and the residue taraxerol, m.p. 280–1° (160 mg 40%). The epitaraxerol thus obtained was found to be identical with the natural sample (m.m.p. and I.R.) thus confirming its structure as epitaraxerol.

The isolation of epitaraxerol is reported now for the first time from nature, although Takeda<sup>5</sup> reported the formation of isotaraxerol, m.p. 267–9°, ( $\alpha$ )<sub>D</sub> +11.9° by reducing taraxerone with sodium and isoamyl alcohol. From its constants, it may, perhaps, be impure taraxerol.

The co-occurrence in nature of 3 $\alpha$  and 3 $\beta$ -hydroxy triterpenes has been revealed more frequently in recent studies. For example, euphol and nerifolol<sup>6</sup> were the first tetracyclic triterpene epimers to be discovered, occurring together in the *Euphorbia* species. Among the pentacyclic triterpenes may be mentioned friedelan-3 $\alpha$  and 3 $\beta$ -ols from *E. antiquorum*<sup>7</sup>, *Quercus champsoni*<sup>8</sup> and *Balanopus australiana*<sup>9</sup> and more recently multiflorenol and epimultiflorenol from *Gelonium multiflorum*<sup>4</sup>. Their frequent occurrence in nature suggests a common origin, possibly a 3-ketone or 2,3-epoxide of squalene, discussed recently by Cotterrell, Halsall and Wriglesworth<sup>10</sup>.

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*Added in the proof.*: Bose and Khashtgir reported the isolation of 3-epitaraxerol from *Macaranga denticulata* Muell Arg. (*Indian J. Chem.*, 1973, 11, 827).

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### 1-(2-QUINOLYLAZO)-2-ACENAPHTHYLENOL (QAAC) AND ITS POTENTIALITY AS A CHROMOGENIC REAGENT

2-HYDRAZINOQUINOLINE was prepared by the method of Perkin and Robinson<sup>1</sup> and acenaphthaquinone required for the reaction was obtained from Fluka A.G. (Purum) and the latter was recrystallised from *o*-dichlorobenzene before use.

2.42 gm of purified acenaphthaquinone was dissolved in warm methanol and a solution of 2.0 gm. of 2-hydrazinoquinoline in 50 ml of 6M HCl was then added with constant stirring. The resulting solution was allowed to stand for some time and then it was neutralized with aq. ammonia solution when a dark brown solid 1-(2-quinolylazo)-2-acenaphthylenol (abbreviated as QAAC) separated out, the yield being 80–90%. The QAAC was recrystallised from methanol and finally it was purified by making use of column chromatographic technique. The purity was further checked by thin layer chromatography. The melting point of QAAC was found to be 195°C. Analysis of the compound (QAAC) (calculated N = 13.0%; C = 78%; H = 4.64% and found N = 13.2%; C = 77.7%; H = 4.42%) corresponded with the molecular formula  $\text{C}_{21}\text{H}_{13}\text{ON}_2$ .

QAAC is a dark brown solid, insoluble in water, dilute acids and alkalis. However, it is soluble

TABLE I  
Colour of various Metal-QAac complexes in different solvents

Metal ion	Water	CCl <sub>4</sub>	CHCl <sub>3</sub>	C <sub>6</sub> H <sub>6</sub>	Ether	1-Pentanol
1. None	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
2. Cd (II)	Red	Red	Red	Red	Red	Red
3. Co (II)	Red	Red	Red	Red	Red	Red
4. Cu (II)	Red	Red	Red	Red	Red	Red
5. Hg (II)	Red	Red	Red	Red	Red	Red
6. Pd (II)	Green	Green	Green	Green	Green	Green
7. Ni (II)	Pink	Pink	Pink	Pink	Pink	Pink
8. Zn (II)	Red	Red	Red	Red	Red	Red
9. V (IV or V)	Orange	Orange	Orange	Orange	Yellow	Orange
10. Nb (V)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
11. Ta (V)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
12. Mn (II)	Red	Yellow <sup>b</sup>	Yellow	Yellow	Yellow	Yellow
13. Rh	Reddish	<i>c</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>
14. Ru	Reddish	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>
15. Pb	Red	Red	Red	Red	Red	Red
16. Fe (III)	Dull red	Dull red	Dull red	Dull red	Dull red	Dull red

(a) Cannot be extracted in the solvent.

(b) Decomposes on extraction.

in acids (pH < 2) to give a cationic species, yellow in colour, which is due to protonation of the nitrogen in the quinoline ring. Also in strong alkalies (pH > 10) it is soluble to give an anionic species, pink to red in colour, which is due to the loss of proton from the hydroxyl group in the acenaphthyl-enol ring. Thus the three forms of QAac involved in acid-base behaviour are related by the equilibria similar to those in PAN and PAR<sup>2</sup>. The order of solubility of this ligand in various solvents is 1-4 dioxane > (chloroform, benzene, carbon tetrachloride) > methanol > ethanol.

QAac forms intensely coloured, water insoluble, complexes with many metals. These complexes can be extracted in various known organic solvents, viz., chloroform, 1-pentanol, benzene, carbon tetrachloride and ether and are soluble in water-miscible solvents such as acetone, ethanol, methanol and 1-4 dioxane. The colours of various metal ion chelates with QAac are listed in Table I.

The metal-QAac complexes are red except those of Pd(II) (green), V(V) (orange) and Fe(III) (dull red). These latter metals can therefore be

determined photometrically without interference by the metals forming red complexes, provided the relative amounts of the interfering ions are not very high. The pH of the solution is of great importance for the formation of chelates and this pH for chelation varies from metal to metal and, therefore, this fact together with their solubility in various organic solvents can be used for micro determination of various metal ions with QAac.

Further work on this potential chromogenic reagent is in progress in our laboratory. One of the authors (Y. L. M.) is thankful to the Council of Scientific and Industrial Research (India) for the award of the Junior Research Fellowship.

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# METAL COMPLEXES OF 4-BENZOYLACETONE-ETHANOLIMINE

A PERUSAL of the literature indicates that no systematic studies have been carried out using ethanolamine Schiff base with benzoylacetone. It has also revealed that the investigation of the complexes of Fe(II), Ni(II), Cu(II), Zn(II), Cd(II), Pd(II) and UO<sub>2</sub>(II) with 4-Benzoylacetone-ethanolimine [HBE] have not been carried out and therefore this work was undertaken.

HBE was synthesised by boiling a mixture of equimolecular proportions of benzoylacetone (3.2 g) and ethanolamine (1.2 g) in dry benzene over a water-bath for an hour in an apparatus provided with water-separator. After the reflux excess of benzene was distilled off and the solid residue was extracted with methanol. The solution was filtered, concentrated and cooled when yellowish-pink crystals of the Schiff base were obtained. These were recrystallized from methanol; m.p. 89° found C 70.11, H 7.19, N 6.71, C<sub>12</sub>H<sub>15</sub>NO<sub>2</sub> requires C 70.25, H 7.32 and N 6.83%. The metal complexes of Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Pd(II) and UO<sub>2</sub>(II) with HBE were synthesised by the method of Yamada *et al.*<sup>1,2</sup>. The pyridine solvates of Fe(II) and Ni(II) complexes were isolated in the solid state by the method reported earlier<sup>3,4</sup>. However, the pyridine solvates of the remaining metal complexes could not be isolated.

Colour, molecular weight, magnetic and spectral data of these complexes are given in Table I.

On the basis of these data the composition of these complexes can be given by the formula [ML<sub>2</sub>] where LH = HBE and M stands for the metal ions. Thus they display 1 : 2 metal-ligand stoichiometry. The magnetic data for the Fe(II) and Ni(II) complexes and their pyridine solvates indicate the presence of 4 and 2 unpaired electrons respectively in these compounds. The molecular weights, 464 and 467 of Fe(II) and Ni(II) complexes respectively thus exclude the possibility of intermolecular association and it is most likely that the intramolecular bond, M-OH, is present in these complexes. This situation may be best represented by an octahedral structure for these compounds. As regards their pyridine solvates it seems that pyridine molecules occupy two coordination positions of the metal ion previously occupied by two -OH groups of HBE. Hence an octahedral structure is suggested for these solvates also. The electronic absorption spectra of Ni(II) complex in dioxan and pyridine show that the frequency correspond to, <sup>3</sup>A<sub>2g</sub> → <sup>3</sup>T<sub>1g</sub>, transition of the Ni(II). It supports an octahedral structure for the Ni(II) complex.

The spectra of Cu(II) complex consists of two absorption bands with peaks at 24,500 cm<sup>-1</sup> and 14,900 cm<sup>-1</sup>. In pyridine nearly similar bands were observed. The band at 24,500 cm<sup>-1</sup> exhibits two

TABLE I  
Colour, molecular weight, magnetic and spectral data of the complexes of HBE

Complex	Colour	Mol. Found	Wt. Calcd.	Molar Susceptibility at 298° K $\chi_m \times 10^6$	Magnetic moment $\mu_{eff}$ in B.M.	No of unpaired electrons	$\bar{\nu}_{max}$ , cm <sup>-1</sup>
Fe (II)	Red	455	464	11,686.72	5.29	4	..
Co (II)	Pink	450	467	8,880.36	4.61	3	14,900
Ni (II)	Dull green	457	467	3,620.68	2.94	2	13,500
Cu (II)	Light blue	485	472	1,470.48	1.87	1	14,900; 24,500
Pyridine solvate of Fe (II)	Rose-red	605	622	12,176.92	5.40	4	—
Pyridine solvate of Ni (II)	Deep green	607	625	3,998.26	3.10	2	—

The Zn(II), Pd(II), Cd(II) and UO<sub>2</sub>(II) complexes were found diamagnetic as expected. These complexes may be represented by the formula [(CH<sub>3</sub>C(O)CHC<sub>6</sub>H<sub>5</sub>C = N(CH<sub>2</sub>)<sub>2</sub>OH)<sub>2</sub>M(II)], where M (II) is the metal ion. Similarly the formula [(CH<sub>3</sub>C(O)CHC<sub>6</sub>H<sub>5</sub>C = N(CH<sub>2</sub>)<sub>2</sub>OH (C<sub>6</sub>H<sub>5</sub>N))<sub>2</sub>M (II)] represents the pyridine solvates of Fe(II) and Ni(II).

shoulders towards shorter wavelength and it is stronger than the other band. The band at  $14,900\text{ cm}^{-1}$  and  $24,500\text{ cm}^{-1}$  may be assigned to the transition,  ${}^2E \rightarrow {}^2T_2$ , of the  $\text{Cu(II)}$ , and intraligand charge transfer respectively<sup>5,6</sup>. The spectra of  $\text{Co(II)}$  complex in dioxan and pyridine consist of only one absorption band with its peak at  $14,900\text{ cm}^{-1}$  which may be assigned to the transition,  ${}^4A_2 \rightarrow {}^4T_1$ . Therefore these complexes seem to possess tetrahedral configuration<sup>7</sup>.

$\text{Zn(II)}$ ,  $\text{Pd(II)}$ ,  $\text{Cd(II)}$  and  $\text{UO}_2(\text{II})$  chelates were found to be diamagnetic and they possess 1:2 metal-ligand stoichiometry. These data may conveniently be explained by assigning a tetrahedral structure for  $\text{Zn(II)}$  and  $\text{Cd(II)}$  complexes, a square planar configuration<sup>2</sup> for  $\text{Pd(II)}$  complexes and an octahedral<sup>8</sup> geometry  $\text{UO}_2(\text{II})$  complex.

Thus in  $\text{Fe(II)}$  and  $\text{Ni(II)}$  complexes the  $-\text{OH}$  group of HBE takes part in coordination and it functions as a tridentate ligand but in the remaining complexes the  $-\text{OH}$  group of the Schiff base does not participate in coordination and it acts as a bidentate ligand.

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#### SOME ENZYMIC STUDIES IN THE LONGITUDINAL SOLE MUSCLE OF VAGINULUS FOOT

THE foot, with its complex pedal musculature forms a basic and adjustable element in molluscan design. But for the function of passive attachment to the substratum in some sedentary forms, the wide variety of locomotory activity patterns exhibited by the foot mirrors most of the principles of locomotion including 'flying' in certain squids. However the chitons and many gastropods retain the primitive wide sole with the production of a continuous locomotor wave. The gastropod pedal locomotor wave has received much attention<sup>1-6</sup> and as in the case of *Helix pomatia*<sup>4,5</sup> there is a direct monotonic pedal wave in the locomotion of vaginulus species. The longitudinal sole muscle of the foot

exhibits a pattern of 8 to 10 dark forwardly moving transverse bands. Though the kinetics and the kinematics of the locomotor wave have been sufficiently worked out<sup>4,5</sup>, the biochemical aspects of the gastropod foot and the musculature therein received a cursory attention. The present report deals with the activities of some of the enzymes in the longitudinal sole muscle of the vaginulus foot.

Large specimens of vaginulus were collected from the local mango grooves and maintained in glass jars containing decaying leaves and wet soil. Just before the assay the specimen was immobilized by cooling in a freezing chamber. The longitudinal sole muscle of the foot was carefully isolated with a bent scissors, washed in gastropod Ringer and chilled to  $0^\circ\text{C}$ . A 2% (W/V) homogenate of the muscle was prepared in 0.25 M sucrose solution using a Potter-Elvehjem pyrex glass homogenizer at a temperature around  $5^\circ\text{C}$ . The activity of some of the dehydrogenases like lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) was assayed following the method of Srikantan and Krishna Murthi<sup>7</sup> and for acetylcholinesterase (AchE) assay the method of Metcalf<sup>8</sup> was used.

The activity levels of oxidative enzymes like SDH and MDH were three fold higher than that of the glycolytic enzyme like LDH (Table I). This

TABLE I

Activity of some of the enzymes in the longitudinal sole muscle of vaginulus foot

(Dehydrogenase activity expressed in  $\mu\text{g}$  formazan/gm tissue/hr., AchE activity expressed in mg acetylcholine hydrolysed/gm tissue/hr. Values are mean of six observations  $\pm$  Standard deviation)

Enzyme	Activity
LDH	280.14 $\pm 46.97$
SDH	707.70 $\pm 246.10$
MDH	735.70 $\pm 279.00$
AchE	518.33 $\pm 36.25$

suggests that aerobic metabolism predominates in the vaginulus foot muscle over the anaerobic metabolism. Since lower glycolytic activity and higher

oxidative enzyme activity characterizes the slow muscle in general<sup>9,10</sup> it may denote that the longitudinal sole muscle of the foot of vaginulus is of a slow muscle type. The muscle had relatively high activity of AchE (Table I). The AchE activity (in terms of mg acetylcholine hydrolysed/100 mg tissue/hr) as quoted by Florey<sup>11</sup> for Nemertini body is 510–899 while it is 31 to 33 for cockroach nerve cord and only 0.3 for the Eusepia muscle. However, in the muscle of *Pontobdella muricata* (Hirudinea) the value was 26–96. In the present material, the AchE activity comes to 50 to 55. This is perhaps the highest value hitherto reported in the literature at least for the molluscan muscle. In view of this, the present material claims a detailed attention pertaining to AchE. The present observation testifies to the prevalence of cholinergic junctions in the sole muscle of the vaginulus foot as in other molluscan slow muscles like anterior byssus retractor muscle of *Mytilus*<sup>12</sup>. This may be summarized that the locomotor wave of the vaginulus foot muscle is aerobically energised and cholinergically generated.

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# THE HAMMERHEAD SHARK, *SPHYRNA LEWINI* (GRIFFITH AND SMITH) FROM THE EAST COAST OF INDIA, WITH REMARKS ON ITS TAXONOMY

THE hammerhead sharks constitute an important component in the drift gill-net catches of the Orissa coast. While working upon material collected recently along this coast, two species were recognised in the commercial catches, viz., *Sphyrna* (*Eusphyrna*) *blochii* (Cuvier) and *Sphyrna* (*Sphyrna*) *lewini* (Griffith and Smith), the latter species hitherto not reported from the east coast of India. Further, an example of *Sphyrna* (*Sphyrna*) *mokarran* (Rüppell), earlier registered as *Cestracion* sp., collected off Puri (Orissa) by the Bengal Fisheries trawler, "Golden Crown" sometime during 1910, was recognised in the Zoological Survey of India collections. The only substantiated record of this species from Indian waters is by Gilbert<sup>1</sup> based on an example from the Krusadai Island (Tamil Nadu). Misra's<sup>2</sup> listing of *S. mokarran* from India is evidently based on Day's<sup>3,4</sup> report from Karachi, then a part of undivided India.

*Sphyrna* (*Sphyrna*) *Lewini* (Griffith and Smith) *Zygaena lewini* Griffith and Smith<sup>5</sup>, p. 640, pl. 50.

*Cestracion leeuwenii*; Day<sup>6</sup>, p. 271 (compiled).

*Zygaena tudes* Day<sup>7</sup> (nec. Valenciennes), p. 720, pl. 188, fig. 4; Day<sup>4</sup>, p. 23; Misra<sup>2</sup>, p. 90.

? *Cestracion oceanica* Garman<sup>8</sup>, p. 158.

*Sphyrna* (*Sphyrna*) *lewini*; Gilbert<sup>1</sup>, p. 37, fig. 10.

**Material.**—1 ex., 491 mm in total length (T.L.), Paradip (Orissa), 18 May 1972, coll. P. K. Talwar, ZSI regd. no. F. 7005/2; 1 ex., 417 mm T.L., Aryipally (Orissa), 30 May 1972, coll. P. K. Talwar, ZSI regd. no. F. 7006/2; 1 ex., 452 mm T.L., Puri (Orissa), 2 June 1972, coll. P. K. Talwar, ZSI regd. no. F. 7004/2.

**Description.**—Head moderately expanded, median indentation (scallop) on anterior margin of head. Outer narial groove absent, inner narial groove extends less than half-way of distance from narial opening to tip of snout. Nares present near eyes, eye-diameter greater than shortest distance from anterior edge of orbit to outer margin of narial opening. Fifth gill slit shorter than first gill slit; first slit situated distinctly behind and fourth slit above insertion of pectoral fin. Dorsal and ventral precaudal pits present.

In percentage of total length: width of head 26.9–28.7, internarial distance 18.2–19.2, snout to symphysis 7.2–7.7, head length 23.4–24.0, snout to first gill slit 17.9–19.5, snout to first dorsal



origin 26.9-30.0, snout to second dorsal 53.8-58.0, snout to pectoral insertion 20.6-22.2, snout to pelvic insertion 42.7-43.6, horizontal diameter of eye 2.6-3.0, length of first dorsal base 9.6-10.6, length of second dorsal base 3.2-4.0, length of second dorsal lobe 4.9-5.3, height of second dorsal fin 2.1-2.6, length of anal base 5.3-5.6, length of anal lobe 3.8-4.6, height of anal fin 3.0-3.3, length of pectoral base 4.8-5.1, length of pelvic base 4.5-5.3, and length of caudal fin 31.4-33.6.

Origin of first dorsal fin slightly posterior to axil of pectoral fin; posterior lobe of first dorsal fin long, about 2.0 height of fin, terminating well anterior to insertion of pelvic fin. Second dorsal fin origin about midway back, above base of anal fin, length of its anterior margin less than length of anterior margin of anal fin, tip of second dorsal lobe reaching 3/4 distance from second dorsal base to upper precaudal pit. Anal fin base more than length of pectoral base and second dorsal base. Pectoral fin 2/3 as broad as long, anterior margin moderately convex. Pelvic fin not falcate, its base equal in length to anal fin base. Caudal fin nearly 1/3 total length.

Teeth broad, smooth; at sides of both jaws oblique, erect teeth in middle of each jaw.

*Colour*.—in alcohol, brownish-grey dorsally shading to white ventrally; a dusky blotch on the upper angle of second dorsal, and on the lower angle of subcaudal lobe and on the tip of the caudal fin.

*Range*.—Circumtropical in distribution. Fairly common in the Atlantic and the Pacific Oceans. In the Indian Ocean it occurs in the Gulf of Aden, south-west coast of India, Burma, Celebes and Java (*vide* Gilbert<sup>1</sup>), Ceylon (*vide* Goonewardene<sup>9</sup>). The present record from the Orissa coast extends its range to the east coast of India.

*Remarks*.—Day<sup>6</sup> listed this species from the south-west coast of India but had no specimens before him. Later, Day<sup>4,7</sup> treated the species as a junior synonym of *Zygaena tudes* (Cuvier). Fraser-Brunner,<sup>10</sup> however, considered Day's (*nec.* Cuvier) *Zygaena tudes* identical with *Sphyrna oceanica* Garman.

Tortonese<sup>11</sup> doubted the validity of *Sphyrna oceanica* Garman and suspected it to be identical with *S. lewini*. Tortonese<sup>12</sup> studied the two extant types of *Zygaena tudes* Valenciennes from the Atlantic Ocean type-localities and concluded that the Indo-Pacific species cannot bear this specific name and the type specimen of *Z. tudes* from Coromandel (India) which is lost, should be associated with *S. oceanica*.

Gilbert<sup>1</sup> treated *Sphyrna oceanica* Garman as a junior synonym of *S. lewini* and considered the

Coromandel type specimen of *Zygaena tudes* as probably identical with *Sphyrna mokarran* (Rüppell). Gilbert<sup>1</sup> described *S. lewini* with an occasional presence of a lower precaudal pit.

An examination of Day's<sup>7</sup> (*nec.* Valenciennes) specimen of *Zygaena tudes* corresponding to Plate 188 (4) (ZSI regd. no. 2330) and a specimen from Mangalore (Mysore State), erroneously identified as *Zygaena zygaena* (ZSI regd. no. F. 5149/2), in our collections clearly shows them to be conspecific with *Sphyrna lewini*. These and the three examples of *S. lewini* from the Orissa coast are characteristic in having a ventral precaudal pit and are likely identical with *Sphyrna oceanica* Garman which is probably a distinct species.

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#### STIMULATION OF SHEEP KIDNEY ALANINE AMINOTRANSFERASE BY $\gamma$ -GLOBULIN, L-GLUTAMIC ACID AND L-LYSINE

IN general, aspartate aminotransferase (AAT) activity of mammalian cells remains fairly steady whereas alanine aminotransferase (AIAT) activity shows wide fluctuations under various physiological conditions<sup>1,2</sup>. AIAT activity of amphibian liver and kidney is known to be sensitive to variations in the protein and amino acid composition of the

TABLE I

Effect of albumin,  $\gamma$ -globulin, glutamic acid and lysine on AlAT and AAT activities in sheep kidney cortex (Enzyme activity expressed in  $\mu$ moles of sodium pyruvate/mg protein/hr)

Enzyme	Parameter	Control	Protein added		Amino acid added	
			Bovine albumin	Bovine $\gamma$ -globulin	L-glutamic acid	L-lysine
AlAT	Mean of $n=6$	3.60	3.00	4.60	6.20	5.80
	*S.D.	$\pm 0.67$	$\pm 0.54$	$\pm 0.55$	$\pm 0.73$	$\pm 0.79$
	% change from control		-16.60	+27.70	+72.20	+61.10
	Significance		† N.S.	$P < 0.05$	$P < 0.001$	$P < 0.001$
AAT	Mean of $n=6$	8.30	6.80	6.50	7.40	7.10
	S.D.	$\pm 1.01$	$\pm 1.43$	$\pm 1.04$	$\pm 0.96$	$\pm 0.80$
	% change from control		-18.00	-21.60	-10.80	-14.40
	Significance		N.S.	$P < 0.05$	N.S.	N.S.

\* S.D. = Standard Deviation.

† N.S. = Not Significant.

extracts<sup>3</sup>. Evidence is presented in this communication to show that sheep kidney AlAT is stimulated by  $\gamma$ -globulin, L-glutamic acid and L-lysine whereas the latter reagents do not affect AAT activity.

Kidneys were excised from healthy sheep and immediately chilled in ice. They were washed in mammalian Ringer<sup>4</sup> solution and finally made into 0.2% W/V homogenates in 250 mM sucrose using a Potter-Elvehjem glass homogenizer. The homogenate was centrifuged at 1000 g for 15 min. at 4°C and the supernatants were used for assay of enzyme activities by the colorimetric method of Reitman and Frankel<sup>5</sup> as described by Bergmeyer<sup>6</sup>. The substances under test were incubated with the enzyme prior to addition of substrate. The results were summarised in Table I. AAT activity was much higher than AlAT activity; however,  $\gamma$ -globulin, L-glutamic acid and L-lysine exerted a stimulatory action only on AlAT. The effect of bovine albumin under identical conditions was marginal.

According to the observations of Nichol and Rosen<sup>1</sup>, the level of AlAT in liver serves as a metabolic barometer, high activity being associated with an increased rate of gluconeogenesis and low activity with the conservation of amino acids for growth. Furthermore, interaction between enzyme protein and other proteins and amino acids<sup>7,8</sup> seems to be confined to AlAT and as such AlAT in

preference to AAT may be subject to regulation by the proteins or amino acids of the milieu.

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# ON THE BREEDING AND COCOONS OF A LITTORAL OLIGOCHAETE *PONTODRILUS* *BERMUDENSIS* BEDDARD.

THE bisexual oligochaetes, after copulation, form characteristic cocoons or egg-cases containing the fertilised eggs, but the formation, structure and the nature of cocoons are not well known in many groups. Little information is available on the breeding and cocoon formation in the littoral species of some Encytraeids and Tubificids<sup>1</sup>.

Reproduction and the formation of cocoons is not fully known in *Pontodrilus bermudensis*, a littoral oligochaete with a world-wide distribution on the warmer waters of both hemispheres. Stephenson<sup>2</sup> and Panikkar and Aiyar<sup>2</sup> have recorded sexually mature individuals during December-January.

*Pontodrilus bermudensis* occurs in large numbers in the local harbour where the salinity conditions widely fluctuate from 5.85 to 34 parts per thousand<sup>1</sup>. Sexually mature worms appear in large numbers at the end of south-west monsoon (October-November) and the shedding of cocoons starts by the end of November and continues till late in May. A few cocoons were collected in June also. The cocoons lie free on the surface layers of the sediment, not exceeding 15 cm in depth. They are also found loosely adhering to the under-surface of pebbles and detritus matter on rocks and they are often found attached to floating twigs and similar structures. During peak periods of cocoon sheddings as many as 750-1825 cocoons were collected from a square meter from the intertidal habitat of the worm.

The freshly laid cocoons are spindle-shaped and green in colour and measure 3-7 mm in length and 2-3.5 mm in diameter (Figs. a, b). With the development of the eggs and the embryos inside, the colour of the cocoon changes to deep pink. When examined under a microscope, the blood vessels and the pumping of blood in the vessels are readily visible through the transparent wall of the cocoon. Most of the dead cocoons were dark and opaque. The number of eggs in the cocoons varied from 1 to 6 but the majority (92%) contained only 1 to 3 eggs. The cocoons are filled with a thick viscous albuminous fluid providing nourishment for the developing worms.

Attempts to induce the sexually mature worms to shed cocoons under laboratory conditions were not successful. The cocoons also failed to hatch under laboratory conditions. Though the worm is tolerant to wide fluctuations of salinity from almost freshwater to near marine conditions, it is interesting

that breeding takes place only when salinity conditions are optimum. Stephenson<sup>2</sup> reported that during the breeding season in Chilka Lake the specific gravity of the water was from 1.008 (10 ‰) to 1.026 (32 ‰). In the local harbour, during the breeding season the salinity ranged from 12 ‰ to 33 ‰. Our observations on the breeding season of *P. bermudensis* in the local harbour correspond with the observations of Stephenson<sup>2</sup> and Panikkar and Aiyar<sup>2</sup> from Chilka Lake and Adyar backwaters respectively.



FIG. a. Photograph of Cocoons,  $\times 4$ .

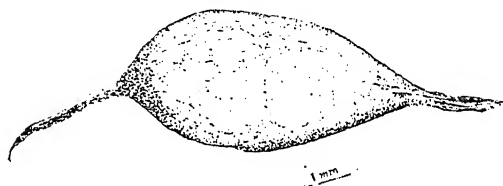


Fig. b. Camera lucida drawing of a Cocoon.

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**POPULATION FLUCTUATIONS OF 3 SPECIES  
OF ANTHOPHILOUS THYSANOPTERA IN  
RELATION TO THE NUMERICAL RESPONSE  
OF THEIR PREDATOR, *ORIUS MINUTUS* L.  
(ANTHOCORIDAE : HEMIPTERA)**

EXTENSIVE information on the impact of density-independent factors on the diurnal, seasonal and annual fluctuations of thrips populations is available, the most significant work being these of Davidson and Andrewartha (1948<sup>1,2</sup>) and Andrewartha and Birch (1954<sup>3</sup>). However, observations on the population fluctuations relating to density-dependent factors among Thysanoptera are scarce as compared with related studies on other insects and mites. Results herein recorded while relating to the numerical fluctuations of populations of 3 species of thrips, viz., *Megalurothrips distalis* (Karny), *Frankliniella dampfi* Priesner (= *F. sulphurea* Schmutz), and *Haplothrips ganglbaueri* Schmutz, and their anthocorid predator *Orius minutus* (L.) also confirm that numerical stability in relation to the carrying capacity is achieved by predation and competition, which are essentially density-dependent and their effect is modified to a greater or lesser degree by weather as well as by the complexity of the environment. That anthocorids appear to be effective predators consuming a good number of adult and young thrips is evident from the predator-prey interaction presented here and this habit coupled with their speed of movement appear to be causative factors in the decline of thrips populations.

*O. minutus* occurs within the inflorescences of *Glyricidia maculata* (Papilionaceae) the flowering season of which extends from middle of February to end of March or beginning of April. All the 3 species of thrips simultaneously occur within the inflorescences, showing varying degrees of dominance in the complete absence of an active predator. The existing environmental conditions remaining constant during the period of observation, the number of thrips per inflorescence showed significant variation (Fig. 1) which was closely followed by variations in the density of anthocorids. Collections were made a short time after the flowers had started appearing and stopped a few days before the end of the flowering season, due to reduction in the number of units taken for examination. Weekly collections for a period of six weeks indicated that the increase in thrips populations was followed by a rise in anthocorid population which in turn resulted in a decline of thrips populations. Reduction in the number of prey caused a steep fall in the number of anthocorids. Although the thrips populations showed a further increase in number, the anthocorids showed no response. A fall in

the thrips population was also evident with the approach of the termination of flowering season. (Fig. 2).

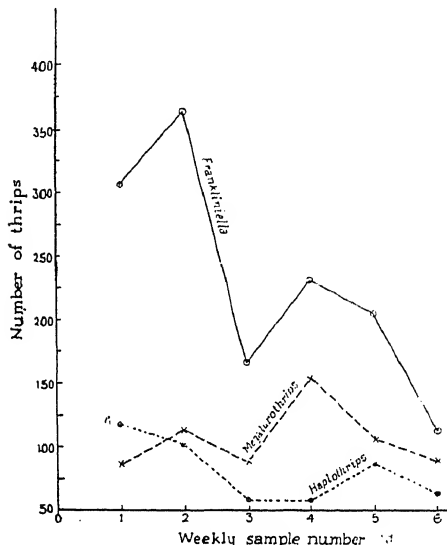


FIG. 1. Graph showing the fluctuations of thrips populations.

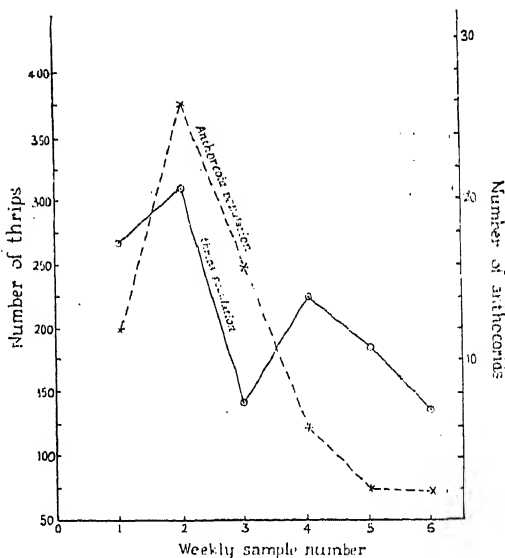


FIG. 2. Graph showing the fluctuations of thrips, and anthocorids.

It is interesting to note that *O. minutus* shows a typical numerical response. The time lag resulting from the delay in establishing itself in the environment is evident from the prolonged time it takes in catching up with the thrips populations as the dominance of the anthocorid was found only in the

second population count recorded. Further studies on the interactions of the thrips-predator populations are in progress.

Thanks are due to Mr. N. Muraleedharan for identifying the anthocorid.

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Madras-34,  
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#### DIFFERENTIAL RESPONSE IN THE CATION EXCHANGE CAPACITY OF THE HOST PLANTS ON PARASITIZATION BY SANDAL (*SANTALUM ALBUM* LINN.)

In an earlier study<sup>1</sup> it was observed that the cation exchange capacity (C.E.C.) of a field-grown sandal gets stabilised irrespective of the spectrum of the associated hosts, and the proximity of the C.E.C. of a host plant to that of sandal appeared to be one of the criteria to indicate whether it was a good host. The study was extended to examine in detail the relationship of cation exchange capacity of a plant and its capacity to serve as a good host to sandal. For this purpose, the C.E.C. of a number of host plants of sandal (*i*) growing alone and (*ii*) having been parasitised by sandal, have been examined. Collection of the roots and determination of C.E.C. were made following the procedure described by Crooke<sup>2</sup>. Young roots of 1 to 2.5 mm thickness, actively serving in mineral absorption, were used for the study. Root samples were taken from mature host plants growing in and around Bangalore. The host plants have been categorised according to the observed fact whether the cation exchange capacity is boosted up, remains more or less the same or becomes depressed on parasitization. The data have been presented in Table I.

It may be noted that the plants mentioned in Category A are in general good host plants, while those in Category B are of a medium quality, and those under Category C are poor. This is understandable because of the fact that the host plant, when being parasitized, needs greater quantities of nutrients, and the rise in C.E.C. on parasitization helps to meet the additional nutritional requirements of the host to nourish the parasite.

With a few differences, the above categorisation is broadly in agreement with the one suggested by Rangaswami and Griffith<sup>3</sup> wherein the number of

haustoria established with the host was taken as the basis. This basis assumes that a greater number of haustoria would provide a greater quantity of nutrition which need not necessarily be true<sup>4</sup>.

TABLE I

		C.E.C. (Milliequivalents per 100 g of dry root; average of 4 different plants in each case)	
I. Sandal		11.5	
II. Host plants		Without parasiti- zation	With parasiti- zation
<b>Category A:</b>			
1. <i>Azadirachta indica</i>	13.0	15.5	
2. <i>Cassia fistula</i>	9.5	14.5	
3. <i>Cassia siamea</i>	12.5	15.0	
4. <i>Dalbergia latifolia</i>	17.0	25.0	
5. <i>Ficus bengalensis</i>	18.0	28.0	
6. <i>Grevillea robusta</i>	19.0	25.0	
7. <i>Pithecellobium dulce</i>	8.5	12.0	
8. <i>Pongamia pinnata</i>	19.0	23.0	
9. <i>Syzygium cumini</i>	12.0	16.0	
10. <i>Wrightia tinctoria</i>	11.0	13.5	
11. <i>Zizyphus mauritiana</i>	13.0	19.0	
<b>Category B:</b>			
1. <i>Acacia farnesiana</i>	20.0	19.0	
2. <i>Acacia polyacantha</i>	13.5	12.5	
3. <i>Anogeissus latifolius</i>	14.5	15.5	
4. <i>Bambusa arundinacea</i>	9.0	9.0	
5. <i>Casuarina equisetifolia</i>	19.0	20.0	
6. <i>Chloroxylon swietenia</i>	14.0	14.5	
7. <i>Lantana camara</i>	22.0	23.0	
8. <i>Prosopis chilensis</i>	14.0	14.5	
9. <i>Psidium guavava</i>	23.0	24.0	
10. <i>Pterocarpus marsupium</i>	22.0	21.0	
11. <i>Semecarpus anacardium</i>	15.5	14.5	
12. <i>Tectona grandis</i>	13.5	14.0	
<b>Category C:</b>			
1. <i>Butea monosperma</i>	18.0	13.0	
2. <i>Dodonaea viscosa</i>	26.5	17.0	
3. <i>Gmelina arborea</i>	19.0	13.0	
4. <i>Melia azedarach</i>	32.5	22.0	
5. <i>Tamarindus indica</i>	33.0	23.5	

Incidentally it may be mentioned that the absolute C.E.C. value of any plant, while growing alone, has no relation to its ability to serve as a good or poor host to sandal.

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SECTORIAL PATTERN IN *EUPATORIUM CONYZOIDES*

REGULAR occurrence of lateral organs in a definite pattern or sequence within a particular species is a good example of organized growth in higher plants. In the flowering plants with opposite and decussate leaf pairs, two principal branching patterns are recognized; sectorial and helicoidal<sup>1</sup>. The former constitutes those cases wherein strongly developed buds lie in two adjacent orthostichies of the four ranks of leaves. The helicoidal on the other hand is characterized by either an axillary bud of each pair at a node being smaller than the other or is entirely missing. Hence two helices can be drawn one through the leaves with larger axillaries and the other along smaller axillaries or empty axils.

In the present investigation the perennial shrub *Eupatorium conyzoides* Mill. a Compositae has been investigated as regards the pattern of branching. To deduce it more than 200 twigs (each with 13–15 nodes at least) were randomly sampled with regard to the size of lateral branches and the leaves subtending them at the same node. The survey was conducted after the break of apical dominance to ensure that at least 13–15 nodes could be analysed in each twig, the extremely young nodes (1–3 or 5) being too small could not be macroscopically surveyed.

The analysis revealed that in more than 90% of the samples sectorial type exists (Fig. 1 A). Only in a few cases a member of the regular sequence was misplaced. The interesting feature accompanying the sectorial pattern was the absence of anisophylly though anisoclady was noted (Fig. 1 B). Often the lateral branches subtended by the sister pair of leaves differed in size by as much as 60 mm.

After the external morphological study of these branches, it was desired to confirm whether the anisoclady observed of the laterals was also present at the time of their inception (unequal primordia being laid down) or is it a case of differential growth due to correlative influences after equal sized primordia are laid down. Apices of terminal shoots were fixed in FAA. Following the customary dehydration techniques serial longi- and trans-sections (10–12  $\mu$  thick) were cut and stained either with safranin-fast green combination or periodic acid Schiff's reagent. The study of the sectioned material revealed that the shoot apex is slightly dome-shaped and measures 75–90  $\mu$  in width. Tunica is distinctly two layered and underneath it is the central mother-cell complex. Cell divisions proceed around the circumference of the apex forming a

kind of collar of tissue linking the leaf bases of a pair of primordia. With the result that in longitudinal sections of the apex the leaf bases of the primordia not in the plane of the section are observed. Young leaf primordia are of equal height from the early developmental stages. Median longitudinal sections of the apex revealed that the inception of the axillary buds can be observed in the axil of the plastochron  $P_2$  primordia (the second youngest pair of leaf primordia). The young bud primordia (60  $\mu$  in width and 54  $\mu$  in height as seen at plastochron  $P_3$  primordia) formed in the axils of a pair of leaf are of equal size (Fig. 1 C). This observation is also supported by the serial transverse sections of the apical bud (Fig. 1 D).

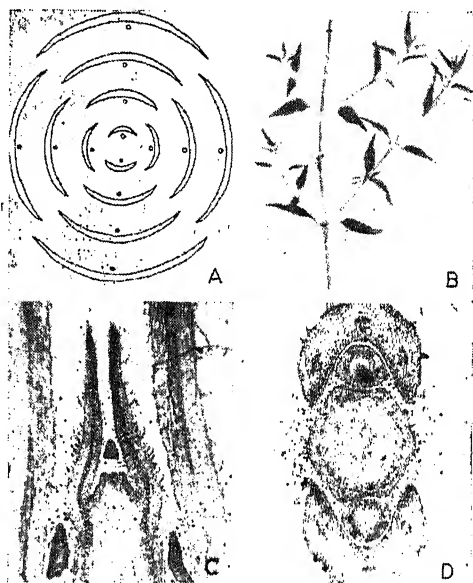


FIG. 1. A–D. A. Diagram of sectorial branching pattern indicating larger buds as (●) and smaller as (○); B. A portion of twig showing anisoclady; C. Median l.s. of the shoot apex showing equal axillary bud primordia in the axils of the  $P_2S$  (the third youngest leaf pair),  $\times 30$ ; D. T.s. of the shoot apex showing in median plane the axillary buds of the  $P_1$  leaf primordia,  $\times 30$ .

Due to subsequent growth the initially equal bud primordia end up in anisoclady. Hence the immediate sub-apical regions of the shoot would offer nodes where transition is established. Surgical experiments under *in vivo* and *in vitro* conditions like excision of one or both axillaries or of leaves subtending them as well as application of various growth hormones are likely to help in understanding the correlative factors that are at play which result in anisoclady in a sectorial pattern. A beginning has been made recently of such studies

including grafting experiments in *Alternanthera* and *Hygrophila* which belong to modified helicoidal type<sup>2,3</sup>.

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#### POLLEN CHAMBER—IN THE OVULE OF *WILLIAMSONIA* CARR.

OCCURRENCE and formation of pollen chamber in the ovule of the bennettitalean plant *Williamsonia* is described for the first time. The material for the present investigation was collected from the Jurassic rocks of Amarjola in the Rajmahal Hills, India. Fructifications of different sizes were studied for the present purpose. Slides were prepared in different planes.

Bennettitalean plants are unique among the Cycadophytes in the structure of their reproductive organs. Male as well as female fertile parts are distinct and different from other cycads. Orthotropus ovules are produced on small, cylindrical seminiferous scales<sup>5,2,3</sup>. Nucellar stalk is long and it terminates into an oval-shaped nucellus<sup>2</sup>. Integument is free from the nucellar stalk but closely adhered with the nucellus<sup>4</sup>. Upper part of nucellus is provided with long, cylindrical cells similar to the one described recently in the ovule of the genus *Cycadeoidea*<sup>1</sup>. The cells are ranging in size from  $48-56 \times 20-22 \mu$  and are filled with some dark staining substance. The peripheral cells are also comparatively larger than those occurring in the central part of the nucellus. The size of peripheral cells reduces gradually, so much so, that in the lower part of nucellus they are like the central cells. The initiation of pollen chamber begins with the degeneration of the apical cells of the nucellus. A fully developed pollen chamber is  $60-65 \mu$  deep (Fig. 1). It is a funnel-shaped structure having the wider part on its outer side. It is surrounded by the elongated cells of nucellus which are filled with dark colouring substance.

However, any pollen grain or microspore-like structure could not be seen in the chamber.



FIG. 1. A fully developed funnel-shaped pollen chamber and the surrounding dark coloured nucellus  $\times 150$ .

The discovery of pollen chamber, and its way of formation by the degeneration of nucellar cell throw light on the relationship of *Williamsonia* with the cycads on the one hand and with the plant like *Ephedra* on the other hand. Presence of pollen chamber in an ovule is certainly a primitive feature but its occurrence in diverse and unrelated group of plants like Cycadales, Ginkgoales, Cordaitales and Ephedrales suggests that this character can hardly be considered of direct phylogenetic importance.

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# ORGANOGENESIS IN ENDOSPERM TISSUE CULTURES OF *CODIAEUM VARIEGATUM* BLUME.

THE endosperm, a triploid tissue in majority of plants, lacks potentiality for organogenesis *in vivo*, and is generally consumed during seed germination. In recent years, with the use of tissue culture techniques, it has been possible to obtain continuously growing tissues and subsequently organogenesis in a few species (Johri, 1971)<sup>1</sup>. This paper describes the *in vitro* responses of endosperm in seed cultures of *Codiaeum variegatum*, a member of the Euphorbiaceae.

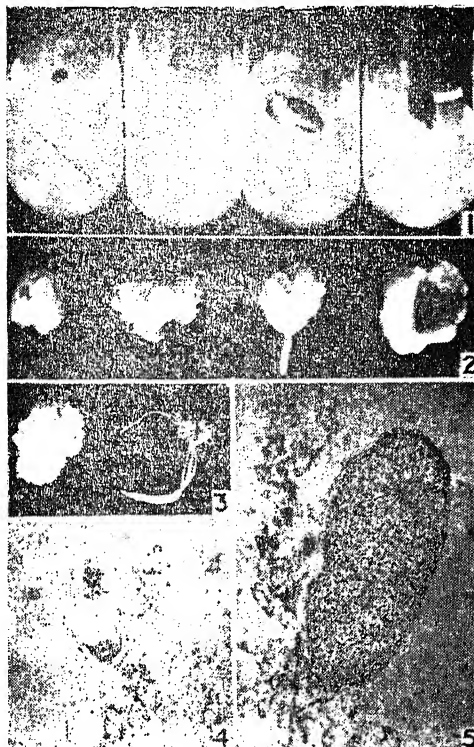
*Codiaeum variegatum* is a very important ornamental plant grown in gardens. The seeds were removed from mature fruits and were surface sterilized in chlorine water for about ten minutes. Following aseptic techniques, the cultures were raised from entire seeds and de-coated seeds on a modified White's agar medium (Rangaswamy, 1961)<sup>2</sup> with 2% sucrose (BM) and also on BM with various supplements like coconut milk (CM), casein hydrolysate (CH), 2,4-D, kinetin, indoleacetic acid (IAA). All the cultures were maintained under diffused daylight (10-20 ft.c.) at about 25°C and 50-60% relative humidity.

At culture, the seeds contained a fully differentiated embryo enclosed by a large endosperm. During the first week of culture on BM, they swelled considerably followed by the emergence of the radicle (Fig. 1). Subsequently normal seedlings were obtained. The plants, thus ensued were dense-green, and after 22 weeks, the leaves showed the variegation, characteristic of the *in vivo* plants.

In about 42% of the cultures, in addition to seed germination, the endosperm yielded a fast growing callus on BM + CM (10%) + 2,4-D (1 ppm) + kinetin (1 ppm) + CH (500 ppm) after four weeks of culture. Initially, the entire endosperm swelled slightly followed by surface proliferation resulting in a mass of callus (Fig. 2). The latter was greenish-white and compact. The actively growing tissue comprised mostly uninucleate cells of diverse sizes and shapes with dense cytoplasm and plenty of starch grains. Occasionally multinucleate cells were also observed. The callus in slightly older cultures comprised several packets of tracheidal cells.

An interesting feature of the tissue ensued from the endosperm was its potentiality for organogenesis. The differentiation of roots (one to many per culture) occurred in several cultures on BM + CM (10%) + CH (500 ppm) + kinetin (1 ppm) + 2,4-D (1 ppm) and BM + CM (10%) + CH (500 ppm) +

2,4-D (1 ppm) in about five weeks (Fig. 3). The roots were normal and had their origin from cells deep inside the callus (Fig. 4). In addition, shoot buds also differentiated independently of roots from the endosperm tissue (Fig. 5).



FIGS. 1-5. Fig. 1. Stages in seed germination on BM × N.S. Fig. 2. Development of callus from endosperm on BM + CM (10%) + CH (500 ppm) + kinetin (1 ppm), × 1.3. Fig. 3. The endosperm callus without roots (at left) and with roots (at right), × 1.5. Fig. 4. Section of cultured endosperm passing through a root primordium. The root is originating from deep inside the callus, × 100. Fig. 5. A shoot bud in section, × 150.

The callus developed from the endosperm was subculturable and continued to differentiate organs. The maximum number of roots differentiated in subcultured tissue was 15, followed by the reduction in the growth of the callus.

Thus the endosperm of *Codiaeum variegatum* proliferates on a complex medium to yield a fast growing callus potentially capable of organogenesis. Similarly, in *Putranjiva roxburghii* differentiation of both roots and shoot buds in a few cultures has been reported<sup>1</sup>. On the contrary, formation of only shoot buds has been observed in *Scurrula pulverulenta* (Bhojwani and Johri, 1970)<sup>3</sup> and *Jatropha pandurafolia*<sup>1</sup>.



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### ON THE MEGAGAMETOPHYTE OF *SESBANIA ACULEATA*—I

*Sesbania aculeata*, Poir., a member of the tribe Galegeae of the Papilionaceae, is studied for the present investigation. Considerable embryological work has been carried out with members of this family. Development of the megagametophyte follows the "Polygonum type". As far as the author is aware there are only two reports of Papilionaceae with increased number of nuclei in the megagametophyte. A nine-nucleate megagametophyte was reported by Roy (1933) in *Dolichos lab lab* and Salgare (1970) in *Phaseolus aureus*. That's why the present observations are noteworthy.

In *Sesbania aculeata* the ovule is bitegmic, crassinucellate and campylotropous. The multiple archesporium is hypodermal in origin. The eight-nucleate, seven-celled megagametophyte is monosporic, with the Polygonum type of development.

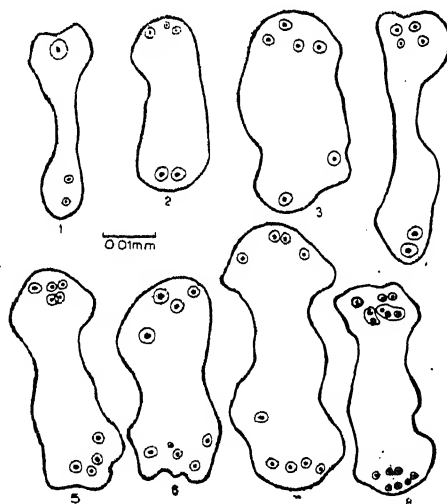
In the present investigation eight types of anomalous megagametophytes were observed. In no case was there formation of egg apparatus and the antipodal cells. The occurrence of the supernumerary nuclei in the megagametophyte may be by the non-simultaneous successive divisions of the nuclei of the megagametophyte.

As can be observed in Figs. 1, 6 and 7 it seems that the chalazal nuclei have divided prior to the micropylar ones.

A three-nucleate megagametophyte (Fig. 1) was observed with a single nucleus at the micropylar end and two at the chalazal end. The former is almost double the size of the latter, indicating the failure of the next division. Another nine-nucleate megagametophyte (Fig. 7) was with four nuclei at the micropylar end and five at the chalazal end. This condition might have arisen by the division of one of the nuclei of the chalazal quartet. Even in one more preparation (Fig. 6), nine nuclei were counted, four at the micropylar end as in Fig. 7 and five at the chalazal end. Here one of the nuclei

of the chalazal quartet divided unequally resulting in a micro-nucleus.

As shown in Figs. 2, 3, 4 and 5 the micropylar nuclei divided earlier than the chalazal. Five nuclei could be counted in one megagametophyte (Fig. 2), two at the chalazal end and three at the micropylar end. Of the three micropylar two are about half the size of the third where the latter might have averted division. The megagametophytes (Figs. 3, 4) show six-nucleate condition, four being situated at the micropylar end and two at the chalazal end, which could have lagged in the next expected division. Another nine-nucleate megagametophyte was also observed (Fig. 5) but here only four nuclei at the chalazal end and five at the micropylar end were seen, a condition, reverse of Fig. 7. Such a case was also reported by Roy (1933) in *Dolichos lab lab* and Salgare (1970) in *Phaseolus aureus*. The nine-nucleate condition might have arisen by the division of one of the nuclei of the micropylar quartet.



FIGS. 1-8. *Sesbania aculeata*. Fig. 1. Three-nucleate megagametophyte, one at the micropylar end and two at the chalazal end. Fig. 2. Five-nucleate megagametophyte, three at the micropylar end and two at the chalazal end. Figs. 3-4. Six-nucleate megagametophyte, four at the micropylar end and two at the chalazal end. Fig. 5. Nine-nucleate megagametophyte, five at the micropylar end and four at the chalazal end. Fig. 6. Nine-nucleate megagametophyte, four at the micropylar end and five at the chalazal end one of them is micronucleus. Fig. 7. Nine-nucleate megagametophyte, four at the micropylar end and five at the chalazal end. Fig. 8. Fifteen-nucleate megagametophyte at the micropylar end three free nuclei, three in one sheath, two in another sheath at the chalazal end seven nuclei. Camera lucida drawings,  $\times 1,500$ .

While coming to the last anomaly the megagametophyte contains fifteen nuclei (Fig. 8), seven, at the chalazal end which might have arisen by the division of three nuclei of the chalazal quartet, while the fourth averted division. At the micropylar end eight nuclei can be observed. Of these three were free nuclei; one cell contained two nuclei, while another cell contained three nuclei. The arrangement of three free nuclei and three nuclei enclosed in a common sheath as well as the unequal size of these nuclei itself indicates that the successive nuclear divisions were not simultaneous.

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#### SOME OBSERVATIONS ON THE FINE STRUCTURE OF LATICIFERS OF *REGNELLIDIUM DIPHYLLUM* L.

LATICIFERS have been rightly described (Cass)<sup>2</sup> as one of the most enigmatic cell types among the angiosperms. It is, therefore, but apt that their morphology and organization were studied both at the light and electron microscopic level since the last century. Contributions of Moor<sup>11</sup>, Andrews and Dickenson<sup>1</sup>, Dickenson<sup>3</sup>, Schnepf<sup>13,14</sup>, Schultze *et al.*<sup>15</sup>, Marty<sup>8,9</sup>, Heinrich<sup>4,5</sup>, and Thureson-Klein<sup>16</sup> have considerably advanced our knowledge about the organization of the various organelles in the laticifers and the loci for the synthesis of the rubber particles.

These studies are, however, restricted only to the angiospermous taxa and *Regnellidium diphyllum* Lind., and *Gnetum* species (see Maheshwari and Vasil<sup>7</sup>), two sole representatives of the vascular plants other than angiosperms, which have been reported to possess laticifers have not been subjected to such an investigation. In this communication, the structure of the laticiferous system in *Regnellidium diphyllum* is described to provide as a basis

for comparison with the similar elements in angiospermous plants.

The material of *Regnellidium* rhizome was obtained from the plants growing at the Heidelberg University Botanical Garden, W. Germany. For light microscopy it was processed in the usual way after fixation either in Craff or FAA. The sections were stained with either safranin-fast green combination or safranin-oil blue mixture as suggested by Richardson (for details see Metcalfe<sup>10</sup>). For electron microscopic observations, thin transverse and longitudinal sections of the material were fixed initially in a combined formaldehyde-glutaraldehyde solution in phosphate buffer at pH 7.2. After fixation these were washed with the same buffer solution several times. These were post-fixed in a 2% osmic acid solution and dehydrated with acetone. The sections were brought in 70% acetone containing uranyl acetate for 1 hr before final dehydration and embedding in Epon-Araldite mixture. Ultrathin sections were cut with glass knives and stained on grids with Reynold's<sup>12</sup> lead citrate for 3 minutes before being viewed and photographed with a Siemens Elmiskop 1 A.

The non-articulated laticiferous elements occur in the subepidermal cells of the cortex, layers of the inner cortex, as well as in the region of the outer phloem (Fig. 1a,b). Viewed with a light microscope, these appear as densely filled cells, possessing numerous granular particles. Their wall is thicker as compared to that of the adjacent parenchymatous cells and they mostly contain a well organized nucleus. In contrast to the observations of Mahabale<sup>6</sup>, fully formed plastids containing starch occur in them, as also proved by the positive PAS reaction.

An electron microscope picture of a single laticifer also reveals that it possesses a thick wall (Fig. 1c) and an abundance of heterogeneously-sized vesicles. The latter are located in a matrix of cytoplasm. The cytoplasm also comprises the usual cell organelles—ER, dictyosomes, mitochondria, and plastids bearing a conspicuous starch grain characteristic of a functioning cell. The nucleus has abundant heterochromatic material (Fig. 1d,e). Furthermore, whereas the mitochondria are mostly restricted along the periphery of the cell lumen, the plastids are distributed all over the cell, including the proximity of the nucleus (Fig. 1e).

Part of this research was carried out at the Lehrstuhl für Zellenlehre, der Universität Heidelberg, Germany. I am grateful to the Alexander von Humboldt Stiftung Bonn, for the award of a Senior Fellowship which made this work possible,

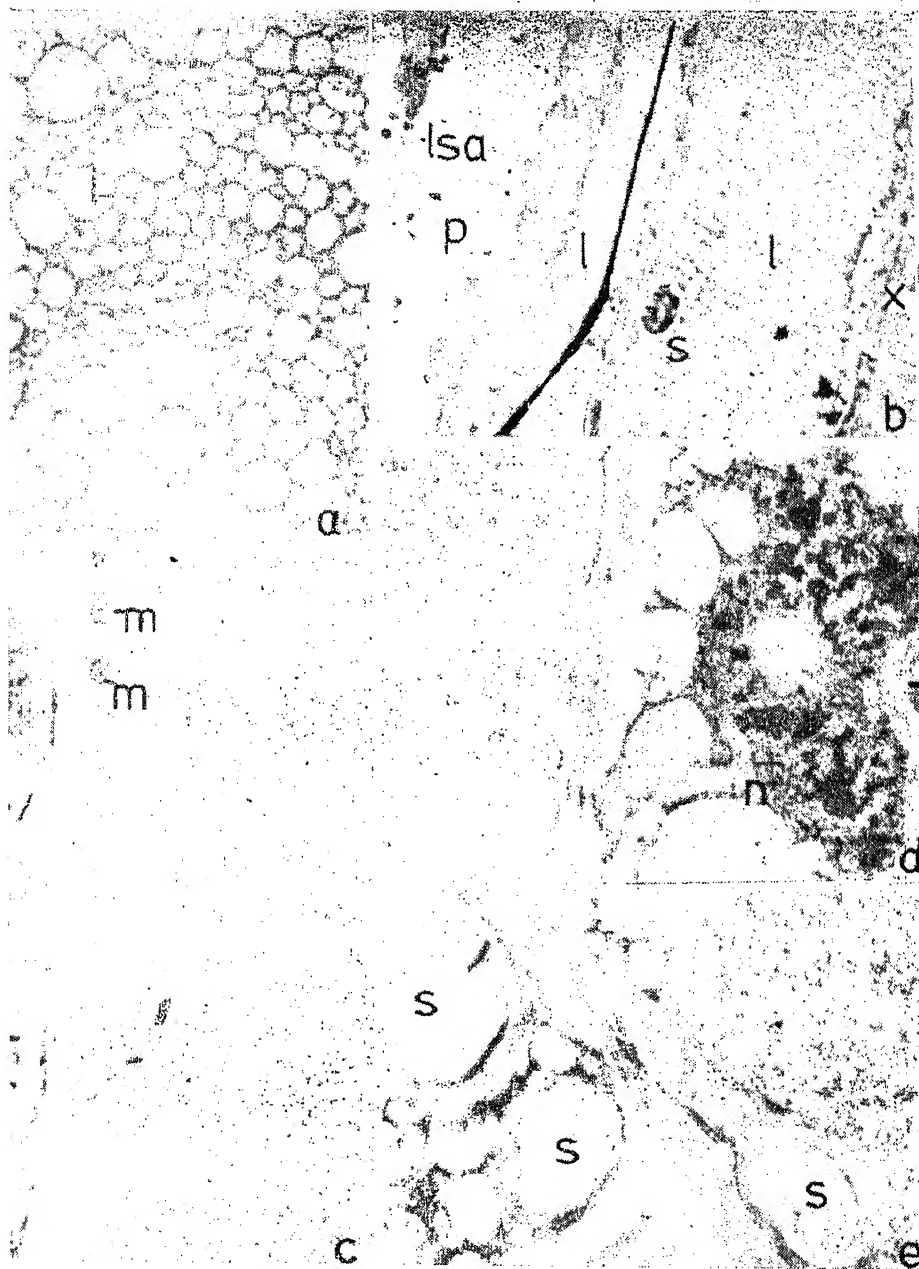


FIG. 1a, *Regnellidium diphyllum*, part of the stellar system as seen in t.s. with a light microscope,  $\times 192$ . (Abbreviations : lsa, lateral sieve area ; m, mitochondria ; p, plastids ; n, nucleus ; S, starch.) Fig. 1b. L.S. Stellar region to indicate the location of the laticifers ; mark the part of the sieve tube with sieve area seen on the left,  $\times 4,500$ . Fig. 1c, d. Distribution and organization of the cell organelles into the laticifer, nucleus of one of the laticifers enlarged to show its well-organized nature, c  $\times 9,000$ , d  $\times 9,800$ . Fig. 1e. Two contiguously placed laticifers possessing plastids with starch,  $\times 9,000$ .

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#### A NEED FOR STRICTER TERMINOLOGY FOR THE SCLERENCHYMATOUS LAYERS IN THE SEED COAT OF MANY PHANEROGAMS

SINCE the turn of this century there has been a resurgence of interest on the tests or seed coat of many phanerogams, especially in the family Fabaceae<sup>1-5,19</sup>, because of its many economic species. The point of histological interest in the seed coat is the occurrence of single, double or sometimes triple layers composed of palisade-like cells with unevenly thickened walls. The correct designation of these layers presents a problem in terminology. Several technical terms like malpighian cells<sup>6</sup>, macrosclereids<sup>7,8</sup>, osteosclereids<sup>20</sup> and descriptive terms: palisade cells<sup>9</sup>, sand-glass cells<sup>11</sup> and hour-glass cells<sup>10</sup> are used while describing their histological identity.

It is evident from the published data that the testa in many seeds constitute a hard coat of one or more sclerenchymatous layers. They appear in regular rows, and composed of cells which often resemble individually macro or palo or osteosclereids but collectively they constitute a tissue. Unlike the idioblastic sclereids they are a part of the whole layer, and further they have no individualistic bizarre growth. Despite their individual resemblance to idioblastic sclereids the most obvious differential features are in being non-idioblastic and tissue forming. These two interesting features are of histological significance from a classificatory point of view because they form a guideline to keep non-idioblastic tissue forming sclereids out of a classification of idioblastic sclereids<sup>12</sup>.

The term sclereid in the exact sense should be restricted to only such cells which are isolated and show a great degree of individuality in size, shape and bizarre growth pattern. In other words, it is an idioblast and constitutes an entity by itself. Despite its monomorphism or polymorphism<sup>14</sup>, it constitutes a distinct solitary sclerosed cell in an organised tissue system. Sometimes they may be loosely disposed in the form of concretions, nests or sclerocysts<sup>13</sup> in an otherwise organised tissue system, and in some instances tissue forming sclereids are found in the form of distinct strands<sup>15-18</sup>.

The concretion sclereids represent an assemblage of loose cells of different base forms and each one differs from the other in shape, size and wall thickness. As a contrast to this in cases of strand formation as seen in leaf, stem and roots they are mainly composed of uniform cells, compactly arranged in rows or strands, and often disposed parallel to leaf surface or margins. In the first, even though they are close to each other, each cell is independent of the adjacent cells, and falls under the category of idioblastic sclereids. In the second, they constitute a tissue composed of homogeneous cells dovetailed into each other, and thereby form a distinct compact strand. So, it is evident that the application of the terms Macro or Osteosclereids *sensu* Tschirch<sup>7</sup> or other descriptive terms to regularly organised sclerenchymatous layers is not appropriate, and in the light of the exact histological sense they cannot be considered as equivalent to idioblastic sclereids. Therefore, the term 'Malpighian layer (cells)' originally applied by Tozzetti<sup>5</sup> (1855) is suggested as the appropriate technical term because this term besides being explicit has a distinct priority over the other dubious terms.

I wish to thank Dr. K. Subramanyam, Director, Botanical Survey of India, for kind encouragement.

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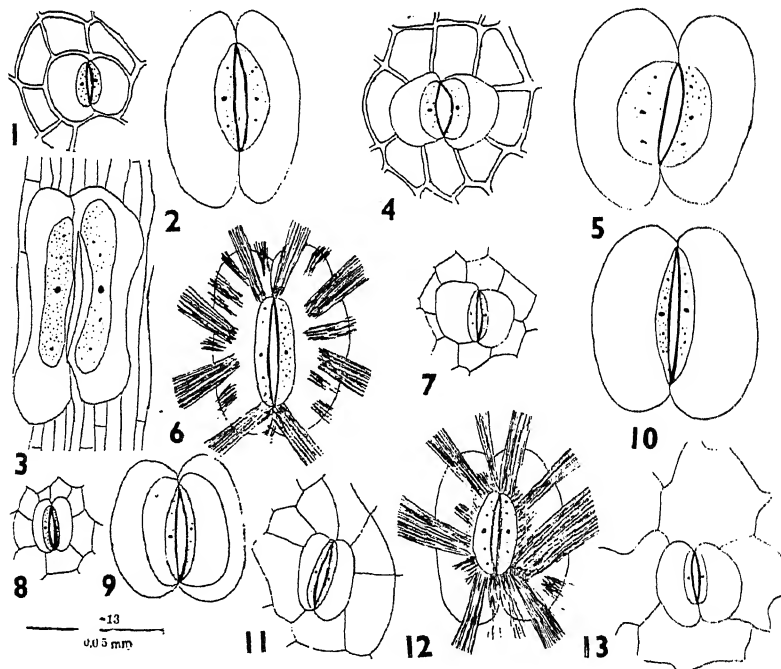
### GIANT STOMATA IN THE FAMILY APOCYNACEAE

GIANT stomata have been recorded in a small number of plants<sup>1,3,4,6</sup>. In the family Apocynaceae giant stomata have been reported only in *Plumeria rubra*<sup>1</sup>, Linn., *Alstonia scholaris*<sup>3</sup> Brown, and *Rauwolfia serpentina*<sup>7</sup> Benth. These are now being

recorded in *Alstonia macrophylla* Wall, *Ichnocarpus frutescens* Br., *Trachelospermum lucidum* K. Schum., *Strophanthus wightianus* Wall, *Tabernaemontana divaricata* Br. and *Aganosma caryophyllata* G. Don by us. Giant stomata observed on the leaves of the above genera are structurally similar to the normal stomata except for their size (Figs. 1–13).

As a rule, normal stomata are restricted to the lower foliar surface but in *Alstonia macrophylla* and *Tabernaemontana divaricata*, they occur on both the surfaces. It has been noticed that giant stomata generally occur on the lower surface though in some cases they have been reported from the upper surface as well. They are generally present on the midrib, veins and venules, occasionally they may occur in association with normal stomata on the areolae.

Giant stomata occurring on the midrib and larger veins have the pore but the guard cells appear to be degenerated and functionless. On the other hand, giant stomata on the areolae have a distinct pore and guard cells, which are surrounded by subsidiary cells. They are comparable to normal stomata and are probably functional. The giant stomata are almost two times larger than the normal



FIGS. 1–13. Figs. 1–2. Normal and giant stomata of *Alstonia macrophylla*. Fig. 3. Giant stoma of *A. macrophylla* on the larger veins. Figs. 4–5. Normal and giant stomata of *Strophanthus wightianus*. Figs. 6–7. Giant and normal stomata of *Ichnocarpus frutescens*, giant stoma showing striations. Figs. 8–9. Normal and giant stomata of *Trachelospermum lucidum*. Figs. 10–11. Giant and normal stomata of *Tabernaemontana divaricata*. Figs. 12–13. Giant and normal stomata of *Aganosma caryophyllata*, giant stoma showing striations.

stomata (Table I). In some giant stomata striations are also seen (Figs. 6, 12).

TABLE I

Name of the species	Size of normal stomata in $\mu$	Size of giant stomata in $\mu$
<i>Alstonia macrophylla</i> Wall	.. 20 $\times$ 13	60 $\times$ 39
<i>Ichnocarpus frutescens</i> Br.	.. 17 $\times$ 7	50 $\times$ 18
<i>Trachelospermum lucidum</i> K. Schum.	17 $\times$ 7	48 $\times$ 16
<i>Tabernaemontana divaricata</i> Br.	23 $\times$ 7	52 $\times$ 22
<i>Strophanthus wightianus</i> Wall	.. 26 $\times$ 7	65 $\times$ 21
<i>Aganosma caryophyllata</i> G. Don.	.. 30 $\times$ 17	65 $\times$ 34

It has been noticed that in *Aganosma caryophyllata* the normal stomata are larger as compared to the other taxa studied and here the giant stomata are twice as large whereas in *Alstonia macrophylla* giant stomata are three times larger in comparison to normal stomata thus there appears to be no correlation in size between the normal and giant

stomata and the ratio seems to vary from plant to plant.

The authors are thankful to the Director, Birbal Sahni Institute of Palaeobotany, Lucknow, for library facilities.

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Lucknow University,  
Lucknow-7, August 6, 1973.

B. S. TRIVEDI.  
NIRMALA UPADHYAY.

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## SHORT SCIENTIFIC NOTES

### A.T.D.C. 12 Computer Program for the Estimation of Genetic Variability, Heritability and Interrelationships of Quantitative Characters in Crop Plants

The estimation of genetic variability; heritability and genetic correlations is of prime importance to the plant breeders and genetists for genetically improving the economic crop species. Though it is quickly needed for decision-making in selection programs the computation of this, through conventional desk calculators, is time consuming and cumbersome. Some quick method, however, is immediately needed.

The purpose of this note is to report a computer program which estimates the above-mentioned genetic parameters. The statistics described by Allard (1960), Burtons (1952) and Hayes *et al.* (1955) have been used in framing this program. Data obtained from a randomised block design with 25 varieties, 3 replications, and 8 characters have been utilized here and same may be read from a

punched tape. The computer output provides the following informations :

1. Analysis of variance table for each character.
2. Analysis of covariance table for each pair of characters.
3. Estimation of heritability, genetic advance at 5% intensity of selection, genotypic coefficient of variation, phenotypic coefficient of variation, environmental coefficient of variation and standard error of the mean and,
4. Estimates of genotypic, phenotypic and environmental correlation coefficients.

The program has been written in 4 K Fortran for the Indian made T.D.C. 12 computer, and has already been used in obtaining the above-mentioned parameters in tomato. Further information concerning details of the program and instructions for its uses may be obtained from us.

We are grateful to Dr. N. K. Anant Rao, Dean, Agriculture; Dr. K. G. Gollakota, Dean, P.G.S.

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#### A New Leaf-Spot Disease of Groundnut from Mysore\*

During the monsoon season (July-September 1973) an unusual leaf-spot disease was observed on groundnut crop (*Arachis hypogaea* L.) under cultivation at Gadag (District Dharwar, Mysore State) by one of the authors (R. R. M.). The infection spots on the leaves were small, sub-circular to irregular, mostly scattered, but few on blades also, dark-brown with clear margins. Critical examination of sections of such spots in the laboratory revealed the presence of a fungus with brownish acervuli, setae, and unicellular cylindrical hyaline, conidia, characteristic of the form-genus, *Colletotrichum* Cda. Sporulation was profuse on such infection spots. The spotting was observed both on young as well as mature leaves. The fungus morphology was: acervuli scattered, ovoid, dirty-brown to brownish, few per spots; setae short, rigid, septate (2-3), slightly broad at base, tapering and blunt at the apex, unevenly distributed in the acervulus, dark-brown, measure  $22.8-38 \times 3.8-4.7 \mu$ . Conidia abundant on host, hyaline, cylindrical with rounded ends, one-celled, measure  $11.4-15.2 \times 3.8 \mu$ .

The fungus on comparison agreed in all respects, of morphological characters and dimensions with *Colletotrichum gloeosporioides* Penz<sup>1</sup>. A perusal of literature indicated that *C. gloeosporioides* Penz. is a new record on groundnut from India<sup>2,3</sup>. Saksena *et al.* (1967) reported a blight disease of this crop incited by another species, viz., *Colletotrichum dematium* (Pers. ex Fr.) von Arx. from Kanpur (U.P.). The material is deposited in the Ajrekar Mycological Herbarium of M.A.C.S., Poona-4, under No. AMH. 1907.

Grateful thanks are offered to Prof. M. N. Kamat for helpful suggestions and to the Director for laboratory facilities.

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Poona-4 (India),	V. G. RAO.
October 17, 1973.	R. R. MALLIKARJUNAIAH.

\* Contribution No. 486 from Department of Mycology and Plant Pathology, M.A.C.S., Poona.

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#### Record of *Chelonus formosanus* Sonan (Hymenoptera : Braconidae), a Parasite of *Spodoptera litura* (Fabricius) from Mysore State

The larvae of the tobacco caterpillar, *Spodoptera litura* (F), feed on a variety of plants and are very injurious to vegetable crops like *Amaranthus*, beet root, brinjal, cabbage, cowpea, sweet potato and tomato in Mysore State. The caterpillars are found in the field almost throughout the year on vegetable and other crops.

During the year 1972 the caterpillars were collected every month from brinjal, cabbage, peas and tomato plants grown around Bangalore and reared on the same host plants in the laboratory to obtain their probable natural enemies.

The larvae collected from July to December 1972 were found parasitised by the Braconid, *Chelonus formosanus* Sonan. The extent of parasitisation varied from 5 to 10%. Maximum number of larvae were parasitised during the month of September.

*Chelonus formosanus* was first described from Taiwan (Formosa) as an egg-larval parasite of *Prodenia litura* (Sonan, 1932). Patel *et al.* (1971) reared this parasite from the same host in Gujarat. The present reporting of *Chelonus formosanus* as a parasite of *Spodoptera litura* is the first record from Mysore State.

The author is grateful to the Director, Commonwealth Institute of Entomology, London, for identifying the parasite.

Univ. of Agricultural Sciences,	P. S. RAI.
Regional Research Station,	
Mandya, Mysore State, September 22, 1973.	

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## REVIEWS AND NOTICES OF BOOKS

**Non-Relativistic Mechanics.** By R. J. Finkelstein. (W. A. Benjamin, Inc., Reading, Massachusetts). 1973. Pp. xvi + 394. Price: Cloth \$17.50; Paper \$9.50.

This book is a successful attempt at giving a unified presentation of classical and quantum mechanics highlighting the many formal similarities existing between them. Unfortunately, much of current graduate-level physics teaching treats mechanics in such a way that much more emphasis and time is given to quantum than to classical mechanics (which may be justified by the greater "current applications" of the former); but, more important, a student usually gets to see the beauty and elegance of the classical theory only at a rather late stage, if ever. This book goes far in remedying this situation, and can lead to a better appreciation of quantum mechanics by bringing out the corresponding structure of classical mechanics at the same time.

The author restricts himself to a discussion of non-relativistic many-particle systems. The book is divided into five chapters. The first chapter recapitulates briefly the main formalisms of classical mechanics: the Newtonian, Lagrangian and Hamiltonian formulations in that order. The ideas of Poisson brackets and canonical transformations are introduced, and the significance of the Galilei group as the underlying space time symmetry group of non-relativistic mechanics brought out. The physical identifications of the generators of this group, their Poisson bracket structure and their conservation, are all discussed.

The second chapter is similarly devoted to a quick review of the traditional formulations of quantum theory. The Heisenberg form of quantum mechanics is obtained from Hamiltonian classical mechanics by the systematic application of the Dirac recipe: replace Poisson brackets by commutators divided by  $i\hbar$ . There follows a discussion of the physical interpretation of the mathematical formalism: operators for observables, vectors for states, the bra and ket notation, probability amplitudes and probabilities, the uncertainty relations, etc. The invariance of the entire mathematical structure under unitary transformations is brought out, and the role of the Galilei group with operators for generators and characteristic commutation relations explained. The passage from the Heisenberg to the Schrödinger picture via a time-dependent unitary transformation is shown.

After these quick reviews of the two kinds of mechanics, the third chapter undertakes a more

systematic and thorough-going examination of the basic structures of the two—bringing out the many deep analogies that exist between them. There is a study of the variational principles underlying classical mechanics, a thorough treatment of the canonical transformation theory, leading up to the Hamilton-Jacobi equation and Hamilton's ideas on wave motion. At this point, the scene shifts to the Schrödinger picture of the quantum theory, and a beautiful exposition of the close similarity between the Hamilton-Jacobi and the Schrödinger equations. Picking up again the idea of variational principles, the transformation functions of quantum theory are identified as the analogues of the classical action, and the Feynman and Schwinger approaches to quantum mechanics are described. Application of these to simple systems like the harmonic oscillator is included. The two concluding chapters apply the general theory to two specific situations—rigid body dynamics and Keplerian motion. Once again, the classical and quantum treatments are given side by side so that one constantly sees the points of similarity and of divergence between the two.

The author states in the preface that this is not a book from which one can expect to learn classical or quantum mechanics for the first time, but one must have a previous acquaintance with these subjects to benefit from this treatment. The reviewer must add that the reader of this book must be prepared to do a fair amount of extra work and thinking to follow through the ideas presented; if this book stimulates him to such effort, he will end up with a really deep and satisfying understanding of mechanics.

N. MUKUNDA.

**Numerical Analysis and Computation: Theory and Practice.** By E. K. Blum. (Addison-Wesley Publishing Co., Reading, U.S.A.), 1972. Pp. xii + 612. Price \$19.50.

This is a very welcome addition to the books on Numerical Analysis. Unlike many other recent books, this book is not just a collection of Computational methods, but develops Numerical Analysis as a legitimate branch of mathematics, bridging the gap between non-constructive mathematics, based on real-number system and constructive system based ultimately on the integers.

The book essentially uses a functional analysis approach. This approach permits the author to deal with measures of error in a unified manner. Yet the book has not sacrificed in any way the numerical



procedures suitable for modern digital computers and the theoretical results are interpreted in terms of computable quantities, wherever possible.

Considerable attention has been paid to scientific computing. Many modern topics such as optimal control problems, boundary value problems, stiff differential equations, Linear programming, spline functions, pseudo-inverse of matrices and Approximation Theory have been treated besides the more conventional topics—finite differences, interpolation and numerical integration.

This book is organized in twelve chapters. Chapters 1 and 2 deal with the basic concepts of analysis and linear algebra. In Chapter 3, a survey of topological vector space is included. Chapters 4, 5 and 6 are respectively concerned with the numerical solution of linear, non-linear equations and eigenvalue problems. Chapter 7 is devoted to approximation theory. The conventional topics of numerical analysis, namely, Interpolation, Numerical Integration and Numerical Solution of Ordinary Differential Equations are dealt with in Chapters 8, 9 and 10. In Chapter 11 the boundary value problems for differential equations are discussed. Chapter 12 deals with constrained and unconstrained optimization problems.

This book can be used as the basis for a two-semester Master's degree Course in Indian Universities for General Applied Mathematics, Engineering and Computer Science. This would help the students to bring in mathematical rigor to each numerical technique they learn.

In addition, this book will also be of great value to a specialist in Numerical Analysis.

E. V. KRISHNAMURTHY.

**Advances in Agriculture (Vol. II).** (Director, Institute of Agricultural Sciences, Kanpur-2, India), 1972. Pp. ii + 139.

The book carries 15 chapters covering several areas of research in the field of Agricultural Science. The material is based on Extramural Lectures delivered by the 15 authors at the invitation of the U.P. Institute of Agricultural Sciences, Kanpur,

during 1972. The authors of each chapter are well-known agricultural scientists in India. They have presented the material in a comprehensive manner covering up-to-date information.

The article on Vegetable Fats by Dr. S. S. Rajan touches on a very important topic of current interest for the country. The article on Physiology of Flowering by Dr. K. K. Nanda gives up-to-date information on basic aspects of flowering in plants. There are six articles on Pests and Diseases of Plants and their methods of control which deal with mostly basic and applied aspects. The two articles on Water Availability and Use bring out the latest information in India on the subject of great importance not only as a field of science, but also of direct concern of the farmers of the country. Dr. B. P. Ghildyal has brought out several theoretical aspects of availability of water to plants. The chapters on Statistics, Quantitative Measurement in Social Research and Extension Methodology are of immediate concern of the scientists as well as Extension Specialists. The last two articles touch on another major issue facing the country, viz., Nutrition and Protein in human and animal life with specific reference to Milk Protein and Nitrogen Balances in animal production.

The 15 articles by the experts have been placed together in one volume bringing certain uniformity in the presentation. The quality of printing and get-up of the book are too poor for the high quality of the contents.

This will be a useful reference book for the Libraries of all the Agricultural and Veterinary Colleges and of the Biology institutions in the country.

G. RANGASWAMI.

#### Books Received

*Complex Analytic Varieties.* By Hassler Whitney. (Addison-Wesley Pub. Co., Reading, Massachusetts), 1973. Pp. xii + 399. Price \$15.95.

*Introduction to Concepts and Theories in Physical Science* (2nd Edition). By G. Holton and S. G. Brush. (Addison Wesley Pub. Co., Reading Massachusetts), 1973. Pp. xix + 589. Price not given.

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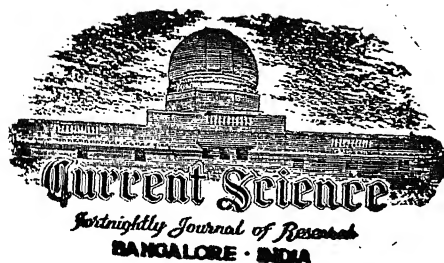
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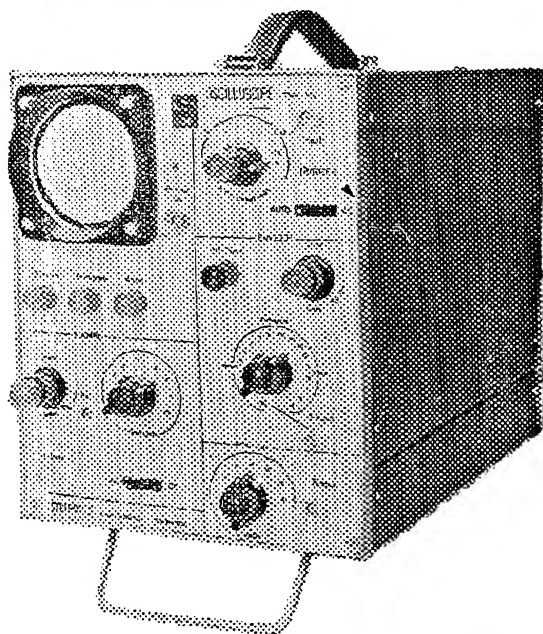
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# SYNTHESIS AND SPECTRAL BEHAVIOUR OF SOME NEW (SUBSTITUTED) BENZOTHIAZOLYL GUANIDINES

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A SERIES of biguanides<sup>1,2</sup> as antimalarials, most notably chlorguanide<sup>2</sup> and daraprim<sup>3</sup> were discovered by Rose and Coworkers. Subsequently, they felt that the activity was due to the N-H group capable of undergoing simultaneous prototropic change with the ring system. This led to the synthesis of guanidine derivatives for antimalarial<sup>4</sup> and antibacterial<sup>5</sup> activities. Recently, some (substituted) benzothiazolyl guanidines<sup>6</sup> have been reported by us exhibiting antiprotozoal activity against *Mycobacterium* 607 and antifungal activity.

In view of the above findings and because of the antibacterial and antitubercular nature of benzothiazolyl guanidines<sup>7,8</sup>, we have prepared some new N-*p*-bromophenyl-N'-(substituted)-benzothiazol-2-yl-N''-(*n*-propyl and *n*-butyl) guanidines by condensation<sup>9</sup> of 2-amino (substituted) benzothiazoles with *p*-bromophenylisothiocyanate in dry benzene and subsequently desulphurisation of the resulting thiocarbamides with alkylamines in presence of yellow lead oxide.

## EXPERIMENTAL

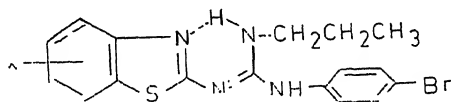
All melting points were taken by the capillary method and are uncorrected. The purity of products was tested by thin layer chromatography

(TLC). Solvent systems used were: Benzene-Ether (3 : 1, *R<sub>f</sub>* 1) and Benzene-Ether (6 : 1, *R<sub>f</sub>* 2). N-*p*-Bromophenyl-N'-(6-methoxy) benzothiazol-2-yl-N''-(*n*-propyl)

**Guanidine 1.**—A mixture of N-*p*-bromophenyl-N'-(6-methoxy) benzothiazol-2-yl-thiocarbamide (3.94 g), yellow lead oxide (4.50 g), *n*-propylamine (1.00 ml) and absolute alcohol (40 ml) was heated in a glass sealed tube on a water-bath at 80–90° for 4–6 hours. After cooling, the sealed tube was broken carefully and the hot black residue was filtered. The filtrate on concentration gave the desired product. It was crystallised from alcohol in beautiful shining crystals, yield 78%, m.p. 118°. TLC : *R<sub>f</sub>* 1 = 0.87. Anal. Calcd. for C<sub>18</sub>H<sub>19</sub>N<sub>4</sub>OSBr : N, 13.37; S, 7.64. Found : N, 13.35; S, 7.68. IR  $\nu_{\text{max}}^{\text{solid}}$  cm<sup>-1</sup> : 3438s, 3200w, 1600s, 1470s. NMR (CDCl<sub>3</sub>)<sup>δ</sup> (J = Hz): 0.96 (3H, t, J = 7.0), 1.63 (2H, m), 3.42 (2H, m), 3.87 (3H, s) and 7.38 for the aromatic protons (7H, m).

Similarly, other (substituted) benzothiazolyl guanidines were obtained by condensation of different (substituted) benzothiazolyl thiocarbamides with *n*-propylamine. The structures and the purity of the compounds are recorded in Tables I and III.

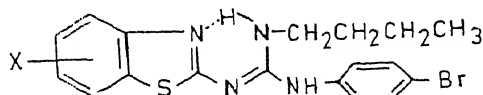
TABLE I



Sl. No.	Substituent X	Molecular formula	Yield (%)	(m.p.) (°C)	Nitrogen (%)		Sulphur (%)		<i>R<sub>f</sub></i> * Values
					Found	Calcd.	Found	Calcd.	
1.	H	C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> SBr	82	98	14.37	14.39	8.12	8.23	.88
2.	4-CH <sub>3</sub>	C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> SBr	63	119	13.86	13.89	7.84	7.92	.89
3.	5-CH <sub>3</sub>	C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> SBr	68	131	13.82	13.89	7.92	7.94	.82
4.	6-CH <sub>3</sub>	C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> SBr	71	141	13.79	13.89	7.84	7.94	.81
5.	4-Cl	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> SClBr	62	126	13.12	13.22	7.52	7.55	.85
6.	5-Cl	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> SClBr	58	128	13.20	13.22	7.57	7.55	.87
7.	6-Cl	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> SClBr	74	120	13.21	13.22	7.49	7.55	.88
8.	6-Br	C <sub>17</sub> H <sub>16</sub> N <sub>4</sub> SBr <sub>2</sub>	68	203	11.92	11.96	6.63	7.84	.87
9.	4-OCH <sub>3</sub>	C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> OSBr	59	110	13.27	13.37	7.53	7.64	.81
10.	6-OCH <sub>3</sub>	C <sub>18</sub> H <sub>19</sub> N <sub>4</sub> OSBr	78	118	13.35	13.37	7.68	7.64	.87
11.	4-OC <sub>2</sub> H <sub>5</sub>	C <sub>19</sub> H <sub>21</sub> N <sub>4</sub> OSBr	66	161	12.92	12.93	7.38	7.39	.86

\* *R<sub>f</sub>* values were measured on developing the TLC plates (adsorbent, silica gel BDH) in Benzene and Ether (3:1) mixture.

TABLE II



Sl. No.	Substi- tuent X	Molecular formula	Yield (%)	(m.p.) (°C)	Nitrogen (%)		Sulphur (%)		$R_f^*$ Values
					Found	Calcd.	Found	Calcd.	
1.	H	$C_{18}H_{19}N_4SBr$	83	85	13.82	13.89	7.82	7.94	.54
2.	4- $CH_3$	$C_{19}H_{21}N_4SBr$	71	103	13.42	13.43	7.65	7.67	.69
3.	5- $CH_3$	$C_{19}H_{21}N_4SBr$	65	101	13.40	13.43	7.68	7.67	.64
4.	6- $CH_3$	$C_{19}H_{21}N_4SBr$	84	108	13.41	13.43	7.58	7.67	.74
5.	4-Cl	$C_{18}H_{18}N_4SClBr$	38	103	12.70	12.80	7.41	7.31	.26
6.	5-Cl	$C_{18}H_{18}N_4SClBr$	49	94	12.65	12.80	7.32	7.31	.83
7.	6-Cl	$C_{18}H_{18}N_4SClBr$	65	114	12.78	12.80	7.30	7.31	.79
8.	6-Br	$C_{18}H_{18}N_4SBr_2$	70	128	11.65	11.62	6.68	6.64	.0
9.	4- $OCH_3$	$C_{19}H_{21}N_4OSBr$	68	123	12.81	12.93	7.37	7.39	.36
10.	6- $OCH_3$	$C_{19}H_{21}N_4OSBr$	74	87	12.91	12.93	7.35	7.39	.46
11.	4- $OC_2H_5$	$C_{20}H_{23}N_4OSBr$	72	173	12.44	12.54	7.30	7.16	.81

\* $R_f$  values were measured on developing the TLC plates (adsorbent, silica gel BDH) in Benzene and Ether (6:1) mixture.

*N*-*p*-Bromophenyl-*N'*-(6-methyl) benzothiazol-2-yl-*N''*-(*n*-butyl) guanidine 2.—A mixture of *N*-*p*-bromophenyl-*N'*-(6-methyl) benzothiazol-2-yl-thiocarbamide (3.78 g), yellow lead oxide (4.50 g), *n*-butylamine (1.2 ml) and absolute alcohol (40 ml) was treated as above to afford the required product. It was crystallised from 80% ethanol into shining needles, yield 84%, m.p. 108°. TLC:  $R_f^2 = 0.74$ . Anal. Calcd. for  $C_{19}H_{21}N_4SBr$ : N, 13.43; S, 7.67. Found: N, 13.42; S, 7.58. IR  $\nu_{max}^{solid}$   $cm^{-1}$ : 3235s, 3105m, 1610s, 1522s. NMR ( $CDCl_3$ )  $\delta$ : 0.97 (3H, m), 1.25 (2H, m), 3.46 (2H, m), 2.46 (3H, s) and 7.49 for aromatic protons (7H, m).

Similarly, other (substituted) benzothiazolyl guanidines were synthesized. Their structures and the purity of the compound are recorded in Tables II and III.

#### DISCUSSION

Varian-A 60D model was used for recording of NMR spectra, Perkin Elmer-257 for IR and a Coleman-Analyser for analyses.

The NMR spectrum (Fig. 1) of the compound 1 in  $CDCl_3$  shows a singlet at  $\delta$  3.87 for the ring methoxy protons, a multiplet at  $\delta$  3.42 for  $-NH-CH_2-CH_2-$  protons, a triplet at  $\delta$  0.96 ( $J = 7.0$  Hz)

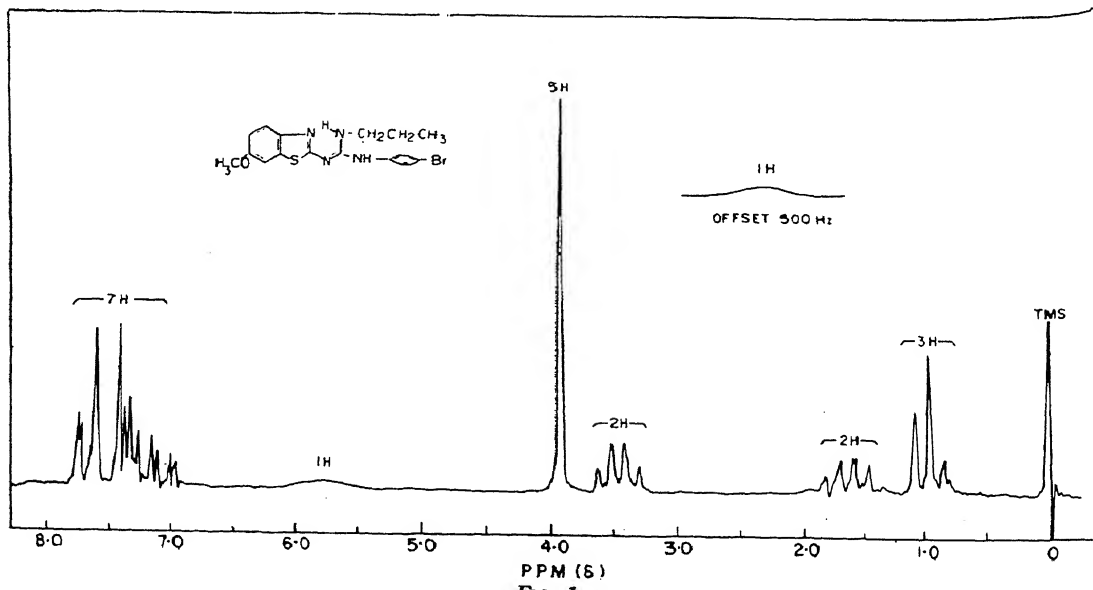
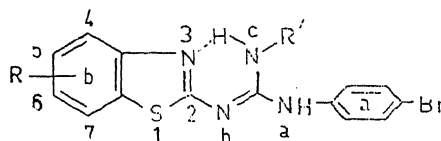


TABLE-III

NMR\* spectra and characteristic  
IR\*\* peaks of



are recorded

Sl. No.	Sub- sti- tuent R	$\delta$ aromatic <sup>a+b</sup>	$\delta$ R <sup>b</sup>	$\delta$ R' <sup>c</sup>	Characteristic IR peaks (cm <sup>-1</sup> )
$R' = \begin{matrix} 1^c & 2^c & 3^c \\ -CH_2-CH_2-CH_3 \end{matrix}$					
1.	5-CH <sub>3</sub>	7.00-7.85 (7H, m)	2.46 (3H, s)	$\delta$ 1 <sup>c</sup> 3.44 (2H, m) $\delta$ 2 <sup>c</sup> 1.55 (2H, m) $\delta$ 3 <sup>c</sup> 0.93 (3H, t) J = 7.0 Hz	3430s, 3165w, 1580m, 1365s,
2.	6-CH <sub>3</sub>	7.00-7.83 (7H, m)	2.46 (3H, s)	$\delta$ 1 <sup>c</sup> 3.45 (2H, m) $\delta$ 2 <sup>c</sup> 1.58 (2H, m) $\delta$ 3 <sup>c</sup> 0.93 (3H, t) J = 7.0 Hz	3438s, 3180w, 1575s, 1365s,
3.	6-OC <sub>2</sub> H <sub>5</sub>	6.75-7.88 (7H, m)	4.28 (2H, d <sub>q</sub> ) J = 8.0 Hz, 1.45 (3H, d <sub>t</sub> ) J = 8.0 Hz	$\delta$ 1 <sup>c</sup> 3.50 (2H, m) $\delta$ 2 <sup>c</sup> 1.63 (2H, m) $\delta$ 3 <sup>c</sup> 0.99 (3H, t) J = 7.0 Hz	3220s, 1615s, 1585s,
$R' = \begin{matrix} 1^c & 2^c & 3^c & 4^c \\ -CH_2-CH_2-CH_2-CH_3 \end{matrix}$					
4.	4-CH <sub>3</sub>	7.12-7.79 (7H, m)	2.58 (3H, s)	$\delta$ 1 <sup>c</sup> 3.47 (2H, m) $\delta$ 2 <sup>c</sup> + 3 <sup>c</sup> 1.54 (2H, m) $\delta$ 4 <sup>c</sup> 0.95 (3H, m)	3235s, 3105m, 1610s, 1522s,
5.	6-Cl	7.22-7.82 (7H, m)	..	$\delta$ 1 <sup>c</sup> 3.48 (2H, m) $\delta$ 2 <sup>c</sup> 1.51 (2H, m) $\delta$ 3 <sup>c</sup> 0.95 (3H, m)	3435s, 3240w, 1620s, 1450s,

\* NMR spectra were recorded in CDCl<sub>3</sub> using TMS as internal standard reference at 44° C. Total number of protons and multiplicity of bands are indicated in brackets.

s = singlet, t = triplet, d<sub>q</sub> = double quartet and d<sub>t</sub> = double triplet.

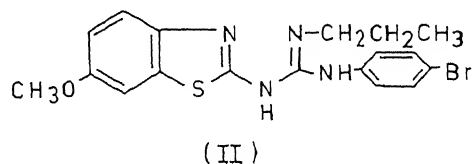
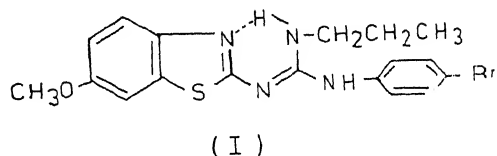
In case of multiplets, the mean positions of the  $\delta$  values are given.

All the compounds gave two NH resonances centred at approx.  $\delta$  5.65 and 10.75 respectively.

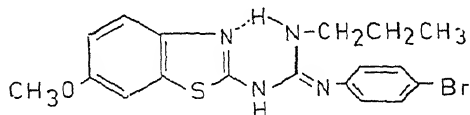
\*\*IR spectra were recorded in nujol.

w = weak, m = medium and s = strong.

for the -CH<sub>2</sub>-CH<sub>3</sub> protons, a multiplet at  $\delta$  1.63 for the -CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>3</sub> protons and a multiplet at  $\delta$  7.38 for aromatic protons. Along with these bands, two >NH resonances have observed at approx.  $\delta$  5.65 and 10.75 respectively. On D<sub>2</sub>O exchange, the two >N-H resonances disappear and the multiplet type band at  $\delta$  3.42 changes into a triplet (J = 7.0 Hz). Therefore, it is evident that the -NH-CH<sub>2</sub>-CH<sub>2</sub>- protons are coupled with an exchangeable proton (J = 5.0 Hz) as well as with an adjacent methylene protons. The above evidences suggest the structure I but not II and IV for the compound 1. The structure III is unlikely



since the structure I is more stable by a more effective conjugation of the planar six-membered ring formed by the hydrogen bonding. The strong IR peak at



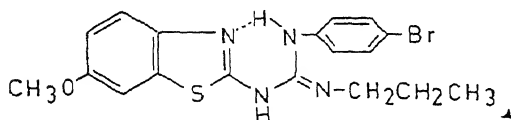
(III)

**Screening Results.**—The microbiological and pharmacological activities of these compounds were carried out at Bristol Laboratories, Syracuse, New York, U.S.A. These compounds were found to be inactive microbiologically but showed remarkable pharmacological activities. Most notably, N-p-bromophenyl-N'-(6-bromo) benzothiazol-2-yl-N'-(n-butyl) guanidine showed CNS depressant, muscle relaxant and anticonvulsant (protection vs. electroshock) as given below:

S. No.*	Area	Microbiological	Pharmacological	MED/MIC	Species
8	Central Nervous System (CNS)	None	Behav. Dep. Muscle Relax. Electroshock	160 mg/kg po 160 mg/kg po 160 mg/kg po	Mouse

MED = Minimum effective dose; MIC = Minimum inhibitory concentration.

\*S. No. corresponds to the S. No. of the compound in Table II.



(IV)

1600  $\text{cm}^{-1}$  which is characteristic of an aromatic type  $\text{C}=\text{N}$ -bond also support the above structure for the compound.

The PMR spectra of the compound 2 in  $\text{CDCl}_3$  also shows along with other normal peaks a multiplet type band at  $\delta 3.47$  for the  $-\text{NH}-\text{CH}_2-\text{CH}_2-$  protons which on  $\text{D}_2\text{O}$  exchange changes into a triplet ( $J = 7.00$  Hz). These facts, therefore, also support the structure for the compound 2 having a skeleton of type I. The strong IR peak at  $1595 \text{ cm}^{-1}$  is also in agreement to its structure.

#### ACKNOWLEDGEMENT

Thanks are due to Prof. G. B. Singh for providing necessary facilities, to the Bristol Laboratories, Syracuse, New York, for screening results and to the C.S.I.R., New Delhi, for financial assistance to one of us (R. S.).

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## OBSERVATIONS ON HORNBLENDE PORPHYROBLASTS IN BASIC GRANULITES AROUND BARAMBA, ORISSA

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**O**CCURRENCE of hornblende porphyroblasts in basic granulites around Baramba ( $20^\circ 27' 30''$  to  $20^\circ 25' \text{ N}$ ;  $85^\circ 20'$  to  $85^\circ 23' \text{ E}$ ). hitherto not reported, is recorded here for the first time.

Basic granulite with porphyroblastic hornblende is sporadically exposed in the close vicinity of the non-porphyroblastic varieties<sup>1</sup>, both types of basic

rock being associated with typical Eastern Ghat rocks. These two varieties are seen in outcrops separated by soil but they can be mapped as single unit based on their field occurrences and absence of lateral variation between the two types.

Porphyroblasts of hornblende (Fig. 1) occur as euhedral to anhedral crystals with sharp outline

and varying in length from 1 cm to 6 cm and width of 1–2 cm. The groundmass shows granulitic texture with plagioclase (andesine-labradorite), pyroxenes, hornblende, quartz, biotite and opaque minerals. Pink garnets have profusely developed in a few cases. Hornblende in groundmass is less in the basic granulite where porphyroblasts are developed.



FIG. 1. Specimen showing hornblende porphyroblasts in basic granulite (dark areas are the porphyroblasts and the rest groundmass)—traced from original specimen.

The porphyroblasts, having the same optical characters as the hornblende in groundmass, are characterised by pleochroism (X—yellowish green, Y—green, Z—greenish brown;  $X < Y < Z$ );  $2V_c = 78^\circ$  to  $84^\circ$ ;  $Z \wedge c = 13^\circ$  to  $18^\circ$ . Both the types of hornblende are secondary. The hornblende porphyroblasts contain numerous inclusions of pyroxenes, plagioclase, garnet and ore minerals. Both the variety lack any preferential orientation. The plagioclase and pyroxene inclusions in the porphyroblasts have the same optical characters as those in groundmass, as well as in the non-porphyroblastic variety. The cleavage planes of the porphyroblast are oblique to those of the included pyroxenes. The pyroxene inclusions have no definite alignment but in few cases the cleavages are parallel.

Hornblende bearing basic granulites invariably contain pyroxenes, whereas a rock with only hornblende (*i.e.*, without pyroxenes) was not traced. Rather, rocks with pyroxenes, lacking in hornblende, were common. Absence of any rimmed structures of hornblende around pyroxenes, inclusions of pyroxenes in hornblende and its (hornblende) growth following the fractures suggest that it has been formed later by metamorphism.

The occurrence of porphyroblasts may be relegated to one of the following possibilities:

- (1) A porphyritic basic intrusive later on metamorphosed.
- (2) Porphyroblastic growth by metasomatism.
- (3) Segregation by metamorphic differentiation.

Field evidences reveal that there is no lateral variation between basic granulites and porphyroblastic varieties. Absence of relict porphyritic texture or corona structure and the comparability in the grain size of hornblende and pyroxenes occurring in groundmass rule out the possibility of the first two hypotheses mentioned above. Of course, the fact that the laws of metasomatism play a role during metamorphism as envisaged by Turner and Verhoogen<sup>2</sup> cannot be ignored. But Turner and Verhoogen<sup>2</sup> and Ramberg<sup>3</sup> are of the opinion that the growth of porphyroblasts is the result of metamorphic differentiation. As such in the present case the formation of porphyroblasts may be attributed to segregation of hornblende grains by metamorphic differentiation which is evidenced by the lack of any original phenocrysts of either pyroxenes or hornblende, presence of a few or few hornblende grains in the groundmass, presence of quartz at the contact of porphyroblast, growth of hornblende along the fractures of pyroxenes, porphyroblasts enclosing virtually all other minerals, even garnet, and finally absence of any corona structure.

Hornblende might have developed due to the interaction of plagioclase and pyroxene<sup>4</sup> under suitable P–T environment. Release of silica attested by occurrence of quartz along the contact of porphyroblast and presence of pyroxene and plagioclase inclusions in hornblende go in favour of the above reaction. The modal composition of four basic granulites (A—non-porphyroblastic without hornblende, B and C—non-porphyroblastic with hornblende, D—porphyroblastic) of the area represented in Table I, reveals that with the increase of hornblende, percentage of plagioclase and pyroxene decreases. This clearly indicates that hornblende has formed at the expense of plagioclase and pyroxene.

TABLE I

	A	B	C	D
qz	x	x	x	1.47
or	1.96	2.31	x	x
plag	47.79	42.71	41.05	38.55
pyr	46.79	40.15	37.81	25.87
Horn	x	10.81	18.19	28.59
gar	x	x	x	0.23
others	3.46	4.01	2.93	5.28



Evidences for primary hornblende could, therefore, not be discernible and its genesis is attributed to retrogression that has led to its formation at a temperature and pressure range of 550–625° C and 3–4 kb. respectively, being the maximum in the facies transitional to granulites<sup>5</sup>; and the porphyroblastic growth by metamorphic differentiation.

The author expresses his gratefulness to Dr. B. Dash, ex-Reader in Geology, for suggesting the topic and helping at different stages of the work. The guidance and timely suggestions of Sri. A. K. Paul, Lecturer, during field work and in preparing this paper are gratefully acknowledged. Thanks are due to Sri. K. N. Sahu and Sri. K. L. Pandya, Lecturers, for their co-operation during laboratory

work. Finally, the author owes his gratefulness to Dr. S. Acharya, Professor and Head of the Department, for providing laboratory facilities.

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## GROWTH OF BARLEY AND WHEAT ENDOSPERM IN CULTURES

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**E**NDOSPERM plays a very significant role in the nutrition and differentiation of embryo. In angiosperms it develops as a result of triple fusion and is mostly triploid. The tissue derived from the culture of endosperm is a homogeneous mass of parenchymatous cells and, therefore, offers a very suitable system for studies on growth and differentiation.

In recent years several attempts have been made to culture immature and mature endosperm, but success has been very limited. So far it has been possible to culture and induce differentiation in the endosperm of some dicotyledonous plants belonging to Euphorbiaceae, Lorantheaceae and Santalaceae (see Johri<sup>1</sup>; Sehgal<sup>2</sup>).

In 1947, LaRue<sup>3</sup> succeeded in establishing cultures of maize endosperm. Since then several workers (Sehgal<sup>4</sup>; Straus<sup>5</sup>; Straus and LaRue<sup>6</sup>; Tamaoki and Ullstrup<sup>7</sup>) made futile attempts to get differentiation and organogenesis in maize endosperm callus. Norstog<sup>8</sup> established continuous cultures from the endosperm of English rye grass. However, he also could not get differentiation from the callus (see also Norstog *et al.*<sup>9</sup>). Trione *et al.*<sup>10</sup> failed to grow the endosperm of wheat. The present work was undertaken to study the morphogenetic potentialities of endosperm in two monocotyledonous plants; barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.).

Ovaries of *Hordeum* and *Triticum* collected 8 days after pollination were surface-sterilized with chlorine water for 7–10 minutes, followed by rinsing in sterile distilled water. The chalazal part of the endosperm was scooped out and planted under aseptic conditions on modified White's basal medium containing 4% sucrose jelled with 0.8% Difco Bacto-agar (WM). The medium was also supplemented with various concentrations of adenine (Ad—20, 40 ppm); autoclaved, coconut milk (CM—10, 20%); casein hydrolysate (CH—0.1, 0.25%); indole acetic acid (IAA—1, 5 ppm); kinetin (Kn—0.5, 1 ppm); yeast extract (YE—0.1, 0.25%); zeatin (Ze—0.5, 1 ppm) and 2,4-dichlorophenoxy acetic acid (2,4-D—1, 5 ppm) either singly or in various combinations. The pH of the medium was adjusted to 5.8 before autoclaving. For each treatment 48 cultures were maintained in diffuse daylight at 25 ± 1° C and 55 ± 5% relative humidity.

In the preliminary experiments endosperms were cultured 4, 6 and 8 days after pollination. The ones excised after 4 and 6 days failed to respond to any of the treatments. Therefore, in subsequent experiments only the endosperms collected from grains 8 days after pollination were inoculated.

In *Hordeum* the endosperm failed to grow on WM or WM supplemented with various concentrations of the above growth regulators, either singly

or in various combinations excepting CH + IAA. On WM + CH (0.25%) + IAA (1 ppm) the endosperm showed the initiation of callusing 10 days after inoculation (Fig. 1 A) in 68% cultures. The growth of callus was slow (Fig. 1 B). When this

callus was transferred to WM + CM (10%) + 2, 4-D (1 ppm) it grew profusely (Fig. 1 C, D) and was yellowish-green in colour. This callus could be easily subcultured on the above medium. Though the callus was cultured continuously for 12 months

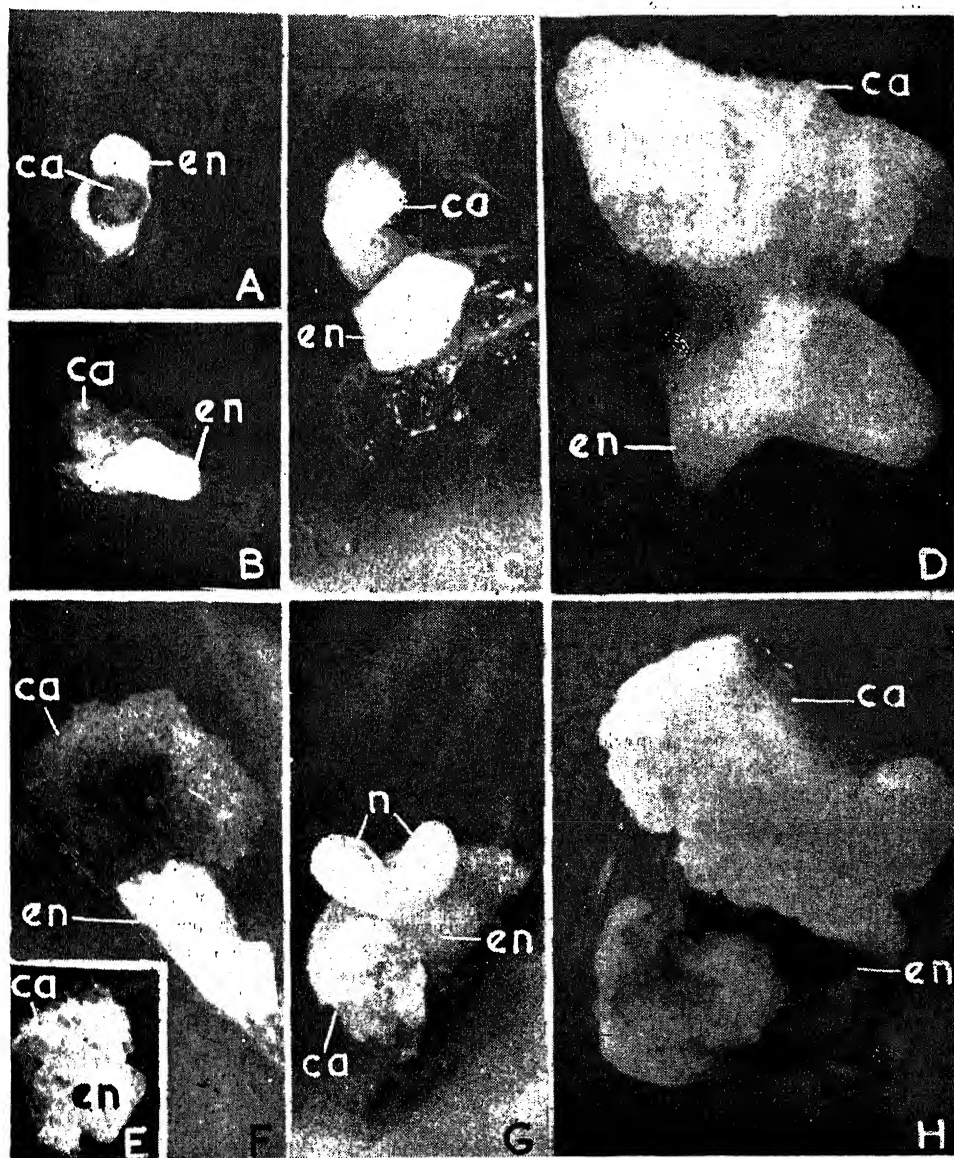


FIG. 1 A-H. A-D. *Hordeum vulgare*, E-H. *Triticum aestivum*. A. 10-day-old culture on WM + CH (0.25%) + IAA (1 ppm), showing initiation of callus,  $\times 4$ . B. Same, 4-week-old,  $\times 4$ . C, D. Endosperm raised for 4 weeks on WM + CH (0.25%) + IAA (1 ppm) subsequently transferred on WM + CM (10%) + 2, 4-D (1 ppm) and grown for 1 and 2 weeks, respectively,  $\times 4$ . E. 1-week-old culture on WM + CM (10%) + Kn (0.5 ppm) + 2, 4-D (1 ppm),  $\times 3$ . F. Same, 2-week-old; note profuse callusing,  $\times 5$ . G. Endosperm raised for 1 week on WM + CM (10%) + Kn (0.5 ppm) + 2, 4-D (1 ppm) and subsequently transferred, on WM + Ad (20 ppm); note two nodular outgrowths which appeared one week after transfer,  $\times 5$ . H. Same, 2 weeks after transfer,  $\times 5$ . (ca, callus; en, endosperm.)

and subjected to different treatments, so far it has not produced any root or shoot.

In *Triticum* the endosperm failed to grow on WM as well as WM supplemented with Ad (20, 40 ppm); CH (0.1, 0.25%); IAA (1.5 ppm); Kn (0.5, 1 ppm); YE (0.1, 0.25%); Ze (0.5, 1 ppm) and 2, 4-D (1, 5 ppm) individually or in various combinations. However, an actively growing callus was obtained on the following combinations: (a) WM + CM (10%) + IAA (1 ppm); and (b) WM + CM (10%) + Kn (0.5 ppm) + 2, 4-D (1 ppm). Of these two combinations, the latter proved to be better because on this medium 84% cultures showed callusing as compared to 62% on WM + CM (10%) + IAA (1 ppm). Callus was initiated one week after culture (Fig. 1 E). It grew rapidly to form a whitish-yellow friable tissue in another week (Fig. 1 F). With the passage of time, proliferation continued but the callus failed to differentiate into plantlets. When the above callus was transferred to WM + Ad (20 ppm) it formed nodular outgrowths in 56% cultures (Fig. 1 G). These outgrowths did not differentiate but callused further (Fig. 1 H)., Trione *et al.*<sup>10</sup> tried as many as 20 different media to culture wheat endosperm, but all their attempts failed.

In the present study I have been able to culture barley and wheat endosperm. However, it has not been possible to get differentiation from the callus. It is concluded that though differentiation from the endosperm of some dicotyledonous plants has been achieved, the production of callus and its differentiation into plantlets from the endosperm of monocotyledonous plants is yet a challenging problem.

I wish to thank Professor H. Y. Mohan Ram for facilities and to Dr. R. N. Chopra for going through the manuscript.

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## LETTERS TO THE EDITOR

### A NOTE ON THE NUCLEAR STRUCTURE PARAMETER AND PERCENTAGE $E_2$ ADMIXTURE FOR THE 57.60 Kev TRANSITION IN $^{127}\text{I}$

THE penetration effect which arises from the penetration of the atomic electrons into the nuclear volume introduces new matrix elements into the conversion electron ejection different from that due to gamma-ray emission. The ratio of this new matrix element to the normal gamma-ray matrix element is defined as the penetration parameter  $\lambda$ . For  $\lambda \neq 1$ , nuclear structure dependent factors will appear in the internal conversion process. The structure effects should be large especially for 1-forbidden  $M_1$  transitions which should have a vanishing gamma matrix element.

The 57.60 Kev transition between  $g_{7/2}$  and  $d_{5/2}$  states is an 1-forbidden  $M_1$  transition with very small  $E_2$  admixture. Up to the date the penetration parameter and the  $M_1$ - $E_2$  mixing ratio have been determined from internal conversion coefficients and particle gamma-ray correlations comparing with the theoretical tabulations of Sliv and Band<sup>1</sup>. But the 1-sub-shell ratios can be measured with an accuracy of 1%. Therefore having in mind the great sensitivity of the ratios to small admixtures the  $E_2$  admixture can be determined with great accuracy. The availability of the recent results of conversion coefficients of Hager and Seltzer<sup>2</sup> and their penetration functions<sup>3</sup> from a new realistic self-consistent field calculations which takes into account the finite nuclear size, hole and exchange effects will be of great use in the present analysis to compare the experimental 1-sub-shell intensity ratios to obtain a more accurate results for the  $M_1$ - $E_2$  mixing ratio ( $\delta^2$ ) and a more precise range for the nuclear structure parameter  $\lambda$ .

By knowing at least two conversion intensity ratios for a given predominantly  $M_1$  transition the penetration parameter  $\lambda$  and the percentage  $E_2$  admixture ( $\delta^2$ ) can be determined using the relation :

$$\delta^2 = \frac{1}{\left(\frac{L_i}{L_k}\right)_{\text{exp}} L_k (E_2) - L_i (E_2)} \\ \times \left[ L_i (M_1) - \left(\frac{L_i}{L_k}\right)_{\text{oxo}} L_k (M_1) \right. \\ \left. + \lambda \left\{ L_i (M_1) B_1 (L_i) \right. \right. \\ \left. \left. - \left(\frac{L_i}{L_k}\right)_{\text{exp}} L_k (M_1) B_1 (L_k) \right\} \right]$$

where  $B_1 (L_{ik})$  are the penetration functions tabulated by Hager and Seltzer and  $L_{ik} (M_1, E_2)$  are theoretical values for conversion coefficients.

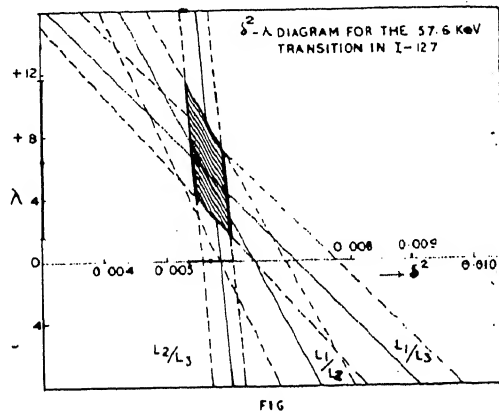


Figure 1 shows the  $\delta^2 - \lambda$  diagram which is used to determine the values of the penetration parameter for  $M_1$  conversion and percentage  $E_2$  admixture for the 57.6 Kev transition. In the same diagram, relative experimental ratio for 1-sub-shell ratios are presented together with statistical errors. (Two statistical error bands around mean value.) The overlapping area is shadowed on the figure and used to determine the experimental region for the values of  $\lambda - \delta^2$ . The most probable pair of values for these quantities in each case is determined by a centre of gravity of the shaded area.

TABLE I

$\delta^2$		$\lambda$	
S. Jha	Geiger	Present work	Present work
$0.6 \pm 0.6$	$0.7 \pm 0.1$	$0.58$	$6.2$
		$+0.03$	$+4.6$
		$-0.04$	$-4.4$

Table I gives the  $\delta^2$  values of Jha<sup>4</sup>, Geiger *et al.*<sup>5</sup> and the present work. The errors associated with the  $\delta^2$  of the present work is very small. This indicates the multipolarity of the 57.6 Kev transition in  $^{127}\text{I}$  as

$$(99.42 \pm 0.3) \% M_1 \\ + (0.58 \pm 0.3) \% E_2$$

The penetration parameter from Table I clearly indicates the penetration effects in the internal conversion process of the 57.6 Kev transition in  $^{127}\text{I}$  and the ratio of the penetration matrix element to the gamma-ray matrix element is  $6 \pm 4$ . The range associated with the penetration parameter is somewhat high and this may be due to the large errors associated with the experimental sub-shell intensity ratios.

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#### PREPARATION OF NEW DERIVATIVES OF $\text{Ph}_3\text{PAuCo}(\text{CO})_4$ WITH SOME BIDENTATE LIGANDS

THIS article is a continuation of work which we have recently published<sup>1</sup>. In that paper we discussed the kinetic investigations of the reaction of  $\text{Ph}_3\text{PAuCo}(\text{CO})_4$  with triphenylphosphine in chloroform as a solvent. We have also reported the

reaction of  $\text{Ph}_3\text{PAuCo}(\text{CO})_4$  with *bis*(diphenylphosphino)ethane, in which we isolated a new compound and proposed an ionic structure for the same.

Now we have reacted  $\text{Ph}_3\text{PAuCo}(\text{CO})_4$  with some new bidentate ligands and have isolated new derivatives. The ligands that we have used are (a) *bis*-(diphenylphosphino)methane, (b) *bis*-(diphenyl arsino)ethane, (c) *bis*-(diphenyl arsino)-methane, (d) *bis*-(diphenyl stibino)ethane, and (e) *bis*-(diphenyl stibino)methane.

The elemental analysis and the C=O stretching frequencies in chloroform and acetonitrile solutions are given in Table I.

The experimental procedure for the preparation of the complexes is the same as previously described<sup>1</sup>. Except for the compound 'A' in the table, all others were obtained as very fine crystalline compounds and therefore they had to be centrifuged and then recrystallized from a chloroform petroleum spirit mixture.

From Table I it is quite interesting to note that the C=O stretching frequencies of all the derivatives in both chloroform and acetonitrile solutions are nearly identical.

We had proposed that this C=O stretching absorption was due to the presence of the species  $\text{Co}(\text{CO})_4^-$  in the solution of the compound. If this assumption is correct then this C=O stretching absorption will not be much affected by the nature of the incoming ligand. The results in the table strengthen the nature and the structure of the com-

TABLE I

Name of the compound			Elemental analysis		C=O Stretching frequencies, $\text{cm}^{-1}$	
			% C	% H	$\text{CHCl}_3$	$\text{CH}_3\text{CN}$
A	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenyl phosphino})\text{ethane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	56.03	3.79	1890	1892
		Found	56.4	3.8		
B	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenylphosphino})\text{methane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	55.63	3.65	1890	1891
		Found	55.49	3.61		
C	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenyl arsino})\text{ethane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	51.62	3.49	1890	1892
		Found	51.50	3.50		
D	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenyl arsino})\text{methane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	51.18	3.35	1890	1892
		Found	51.20	3.21		
E	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenyl stibino})\text{ethane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	47.60	3.22	1890	1891
		Found	47.51	3.10		
F	$[\text{Ph}_3\text{PAu}-\text{bis}(\text{diphenyl stibino})\text{methane}]^+ [\text{Co}(\text{CO})_4]^-$	Calcd.	47.17	3.09	1890	1891
		Found	47.27	3.13		

plex which we had suggested in the previous article<sup>1</sup>.

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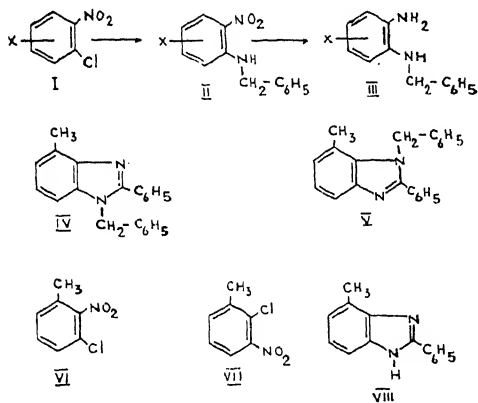
R. T. SANE.  
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## REACTION OF *o*-CHLORONITROBENZENES WITH BENZYLAMINE

ONE of the unambiguous methods of synthesis of 1-benzyl benzimidazoles<sup>1,2</sup> involves the reaction of an *o*-chloronitrobenzene (I) with benzylamine. reduction of the resulting benzylamino nitrobenzene (II) and cyclisation of N-benzyl-*o*-phenylenediamine (III) thus obtained, by condensation with an aromatic aldehyde in the presence of an oxidising agent<sup>2</sup> or with an aliphatic acid by Phillips method<sup>3</sup>.

During our studies on the formation and reactivity of benzimidazole ring system we have adopted this method for the synthesis of 1-benzyl-2-phenyl-4-methyl and 7-methyl benzimidazoles (IV and V) starting from the isomeric chloronitrotoluenes (VI and VII).



Condensation of 3-chloro-2-nitrotoluene (VI) with benzylamine in the presence of fused sodium acetate around 200° gave, however, in addition to the expected N-benzyl-2-nitro-3-aminotoluene, 4 (or 7)-methyl-2-phenyl benzimidazole (VIII) in good yield. Likewise, condensation of the isomeric 2-chloro-3-nitrotoluene (VII) with benzylamine has yielded a good amount of (VIII) along with N-benzyl-2-amino-3-nitrotoluene.

In the alternate synthesis of these N-benzyl-*o*-nitro anilines by the benzylation of *o*-nitroanilines with benzyl chloride<sup>2</sup>, such direct formation of

benzimidazoles has never been noticed. As such in the present reaction it appears that part of the *o*-chloronitrotoluene is involved in some side reaction with benzylamine prior to the expected nucleophilic displacement of the activated chloro group. To study the generality of this reaction the parent *o*-chloronitrobenzene has been subjected to reaction with benzylamine under identical conditions. Once again 2-phenyl benzimidazole is the major product along with N-benzyl-*o*-nitroaniline.

These results indicate that during the reaction of an *o*-chloronitrobenzene with benzylamine, a one step benzimidazole formation appears to predominate. This appears to be the first case of direct formation of benzimidazole ring system in the reaction of a nitrobenzene, containing a labile ortho substituent, with an aralkylamine.

Full details and the mechanism of the reaction will be published elsewhere.

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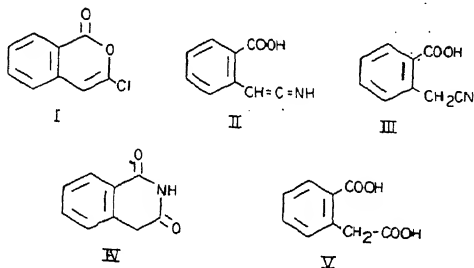
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## REACTION OF 3-CHLOROISOCOUMARIN WITH AMMONIA

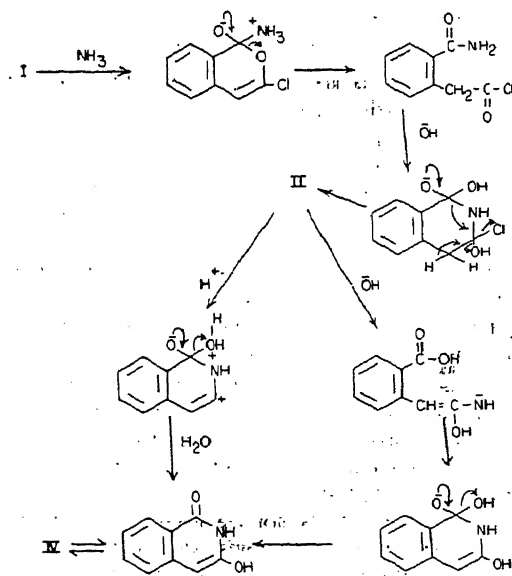
3-CHLOROISOCOUMARIN<sup>1,2</sup> (I) reacts with ammonia in an interesting manner. When an ethanolic solution of (I) (1 g in 15 ml) was refluxed with liquor ammonia (20 ml) for 3 hr and the resulting solution after concentrating to a small bulk was acidified, a crystalline acid, 2-carboxyphenyl ketenimine (II) was obtained. [Crystallised from benzene-petrol mixture as colourless needles, m.p. 116–18°; yield 0.8 g. Found: C, 66.5, H, 4.7, N, 8.3; C<sub>10</sub>H<sub>7</sub>O<sub>2</sub>N requires C, 67.0; H, 4.3; N, 8.7%; U.V.  $\lambda_{\max}^{\text{MeOH}}$  226, 275 nm (log  $\epsilon$  4.09 and 3.36)]. The same acid (II) was also obtained when 3-chloroisocoumarin (I) was mixed with liquor ammonia, heated on boiling water-bath for 3 hr, excess of ammonia evaporated off and then the mixture was acidified.

The fact that (II) is not the isomeric 2-carboxyphenyl acetonitrile<sup>3</sup> (III) was shown by m.m.p. with the authentic specimen of the latter that showed a lowering of 16°. Evolution of NH<sub>3</sub> took place on refluxing (II) with aq. NaOH (10%) and the solution on acidification furnished homophthalic acid (V), but when (II) was warmed with aq. NaOH (10%) on water-bath at 40–50°, for half

an hour and then acidified, it isomerised to homophthalimide (IV) [crystallised from ethanol, as



needles, m.p. 234–35°; Found C, 67.5; H, 4.0; N, 8.3%;  $C_9H_7O_2N$  requires C, 67.1; H, 4.4 and N, 8.7%; U.V.  $\lambda_{max}^{MeOH}$  240, 290 nm (log  $\epsilon$  4.05 and 3.21). The isomerisation of (II) to (IV) also took place when (II) was heated with  $H_2SO_4$  (67%) on water-bath for half an hour. The isomerised substance gave homophthalic acid (V) on refluxing with aq. NaOH, with evolution of  $NH_3$ . The imide was identified as homophthalimide (IV) by m.m.p. with the authentic specimen (m.p. 234–35°), prepared as given in lit.<sup>5,7</sup>. Homophthalimide (IV) was also obtained by passing dry ammonia gas in molten homophthalic anhydride<sup>6</sup> (140–50°), m.p. and m.m.p. 234–35°.



The structure of 2-carboxyphenyl ketenimine (II) followed from the reactions described above and also from its IR and NMR spectra. IR (KBr) showed bands at 3400 (broad,  $=N-H$ ), 2210 (sharp

$-C=C=N-$ ), 1690 ( $>C=O$ ) with a shoulder at 1650 (may be  $>C=C<$ ), 3260–2500 (jagged bands,  $-OH$  of  $COOH$ ), 1600, 1490, 1410, 1280, 1230, 765 and  $755\text{ cm}^{-1}$  ( $\alpha$ -disubstituted benzene). NMR ( $CDCl_3$ ) gave signals for aromatic protons at  $\delta$  8.4 (1H, splits at peak  $m$ , the deshielded proton *ortho* to  $-COOH$ ), 7.4 (3H, *m* but appearing as  $dJ=14$  Hz, the three remaining aromatic protons). Two singlets at  $\delta$  3.9 and 2.16 accounted for  $-CH$  and  $NH$  protons respectively of  $-CH=C=NH$ . Interesting and significant point about them is that the signal at  $\delta$  3.9 integrated for little more than 1H and that at 2.16 for less than 1H and on the base of the signal at  $\delta$  3.9 a very small peak ( $\delta$  4.0) is seen suggesting some isomerisation or exchange of proton.

The formation of 2-carboxyphenyl ketenimine (II) and its isomerisation to homophthalimide (IV) in presence of acid as well as base may be explained by the mechanism given above. It may be pointed out that replacement of heterocyclic  $-O-$  by  $-NH-$  by the action ammonia on isocoumarins that give corresponding isoquinolones, is a well-known reaction and that is considered to proceed through the formation of intermediate amide<sup>4</sup> similar to that given in the above mechanism. It is the  $-Cl$  that alters further the course of reaction because of its tendency for being readily eliminated.

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INTERFEROMETRIC ESTIMATION OF METHYL  
AND ETHYL ALCOHOLS IN BIOLOGICAL  
MATERIAL

ETHYL alcohol estimation in blood, both from living subjects and in post-mortem samples, is a routine procedure in forensic laboratories. The simultaneous determination of methyl and ethyl alcohols in post-mortem samples, viscera, blood, etc., also becomes necessary when fatal poisoning has occurred due to consumption of liquor containing or contaminated with methyl alcohol. Such tragedies on large scale have occurred a number of times in our country.

The determination of ethyl and methyl alcohols in biological samples, though possible presently by gas chromatography<sup>1</sup>, is still largely done by chemical procedures. The combined alcohols are quantitated by Widmark's diffusion-oxidation method<sup>2</sup> or a suitable modification thereof<sup>3,4</sup>. Methyl alcohol is oxidised to HCHO (permanganate-phosphoric acid)<sup>5</sup> and subsequently quantitated using Schiff's reagent<sup>1</sup> or chromotropic acid<sup>6</sup>. Ethanol is then evaluated by difference.

In the estimation of ethanol alone, it is desirable to confirm the values by an independent physico-chemical procedure for better specificity of the results. When both methanol and ethanol are present, it is still more advantageous to confirm the values by another independent method. Widmark method is also reported to be associated with spuriously higher values of apparent alcohol<sup>7</sup>, while in methanol estimation colorimetrically it is essential to perform exact controls with MeOH, added to the biological material<sup>1</sup>. In the present work an interferometric method of estimation of ethanol or methanol-ethanol from viscera samples is described. Bock's interferometric work<sup>8</sup> on estimation of 'apparent' alcohol in normal blood may be cited here.

Zeiss laboratory interferometer was used with liquid cells of 4.0 cm path length. Measurements were made against distilled water. The liquid cells were immersed in a water-bath at room temperature (air-conditioned at  $\sim 27^\circ\text{C}$ ). The compensator readings,  $\Delta N$  ( $\propto \Delta n$ ), have been used as such in calculations in place of actual  $\Delta n$  values. The distillates from "blank" viscera samples (known to contain no alcohol and free from other toxic material; distillation in all glass apparatus) showed high blank values on the interferometer. This could be satisfactorily removed by mixing the distillate with solid KOH on a warm water-bath ( $\sim 10^\circ\text{C}$ ) for  $\frac{1}{2}$  hr followed by re-distillation. This procedure was followed for analytical samples. Though an activated alumina column was found

to purify the blank distillates, it could not be adopted for analytical samples in view of marked loss of alcohol on the column above 100 mg% concentration.

Interferometric estimation of ethyl alcohol, when alone in biological material, involves direct reading of the concentration from the linear calibration curve (concentration v.s.  $\Delta N$ ). Typical recovery results are presented in Table I.

TABLE I

Sample	EtOH added mg%	Recovery mg%	Error
Liver, etc. ..	200	186.5	-6.7%
Stomach and intestines	400	378.5	-5.4%
Liver, etc. ..	600	574.0	-4.0%

TABLE II

$C_1$ (MeOH mg%)	$C_2$ (EtOH mg%)	$\Delta N$ (Reading)
0.0	500	7.09
50.0	428.15	6.21
100	356.25	5.40
200	212.85	3.60
300	68.75	2.00
349.9	0.0	1.20

The magnitude of the negative error by the present method is less than that of the positive error by chemical (oxidation) method. This is to be preferred on both counts, sign and magnitude.

It was possible to determine ethanol-methanol concentrations ( $C_2$  and  $C_1$  respectively) when present together in viscera (10 g sample), from interferometric and oxidation data, the latter by Mahál's technique<sup>4</sup>. Simultaneous equations

$$\Delta N = C_1 \Delta n_1 + C_2 \Delta n_2 \quad (1)$$

and

$$T = C_1 t_1 + C_2 t_2 \quad (2)$$

were set up and readily solved for  $C_1$  and  $C_2$ .  $\Delta N$  is the observed interferometric reading and  $T$  the observed titre. Additivity relation (1) was confirmed by taking MeOH-EtOH mixtures in the range 0-500 mg%. The titre of each mixture (Table II) was held constant.

$$\text{Let } C_0 = 1.437_5 C_1 + C_2 = \text{constant (held at 500 mg\%)} \quad (\text{note } t_1/t_2 = 1.437_5)$$

$$\Delta N = C_1 (\Delta n_1 - 1.437_5 \Delta n_2) + C_0 \Delta n_2 \quad (3)$$



$\Delta N$  should be linear with reference to  $C_1$  and is observed so (Fig. 1) whence  $\Delta n_1$  and  $\Delta n_2$  can be calculated for further analytical work.

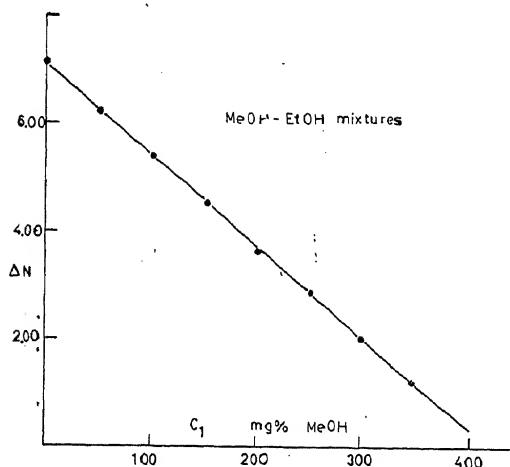


Fig. 1

The recovery data using the procedure described above is given in Table III.

TABLE III

No.	mg% added to viscera	mg% found	Error %
1	EtOH-400	382.9	- 4.3
	MeOH-100	85.9	(- 14.1)
2	EtOH-325	303.7	- 6.5
	MeOH-175	164.3	- 6.1
3	EtOH-250	232.8	- 6.9
	MeOH-250	238.1	- 4.8
4	EtOH-175	188.7	+ 7.8
	MeOH-325	316.8	- 2.0
5	EtOH-100	100.0	0.0
	MeOH-400	378.1	- 5.5

The results obtained by the present method are reasonably accurate and better than those obtained by the usual alternative (oxidation/colorimetric) method.

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### NEUTRAL CONSTITUENTS OF *ALBIZZIA LEBBECK*

*Albizzia lebeck* Benth. (Leguminosae, *shirisha*) is a large tree grown throughout India. The plant has been described to have various medicinal uses in the indigenous system of medicine<sup>1</sup>. Report<sup>2</sup> about the successful use of the bark and flowers of this plant in clinical cases of asthma prompted us to undertake the present investigation.

Though a lot of work<sup>3-4</sup> on the saponins of *Albizzia lebeck* and its other species is on record, no work on the neutral constituents of the bark has yet been reported. The present communication describes the isolation and characterisation of friedelin and  $\gamma$ -sitosterol from the bark of this plant. Powdered bark of *A. lebeck* (4.5 kg) collected locally was extracted with rectified spirit by cold percolation. The alcoholic extract was absorbed in paper pulp and extracted with pet. ether (60-80°) in a soxhlet. The pet. ether extract (9.0 g) was dissolved in ether and fractionated into neutral (6.4 g), acidic and phenolic components. The neutral fraction contained seven constituents as revealed by thin layer chromatography. By detailed chromatographic resolution over Brockmann alumina four compounds have been isolated, of which two have so far been chemically characterised. Elution of the column with pet. ether : benzene (9 : 1) mixture furnished friedelan-3-one (0.18 g), m.p. 260-262° (EtOAc) (lit.<sup>5</sup> m.p. 263-263.5°),  $R_f$  0.6 (SiO<sub>2</sub> TLC, Benzene; Ac<sub>2</sub>O : H<sub>2</sub>SO<sub>4</sub> : EtOH, 5 : 5 : 90 as spraying reagent), C<sub>30</sub>H<sub>50</sub>O (M<sup>+</sup> 426). The infrared spectrum of the compound shows bands at 1710 cm<sup>-1</sup> (a six-membered carbonyl). The NMR spectrum exhibited signals due to quaternary methyls [ $\delta$  0.71 (6 H, s), 1.01 (6 H, s), 1.1 (9 H, s)] and a secondary methyl [ $\delta$  1.45 (3 H, d, J = 4 Hz)] with a multiplet at  $\delta$  2.2 (CH<sub>3</sub>-CH-C-CH<sub>2</sub>-CH<sub>2</sub>-). The compound gave a D.N.P. derivative, m.p. 295-296° (benzene) lit.<sup>5</sup> m.p. 297-299°. The mass spectrum of the parent ketone, in addition to the molecular ion peak at m/e 426 (100%) showed significant fragment ions at m/e 411 (M-Me, 28%), 341 (13%), 302 (49%), 273 (74%), 246 (37%), 232 (45%), 218 (55%), 205 (66%), suggesting its identity with friedelan-3-one<sup>6</sup>.

The pet. ether : benzene (7 : 3) mixture yielded  $\gamma$ -sitosterol (0.03 g), m.p. 147-151° (EtOH) (lit.<sup>7</sup> m.p. 147-148°),  $R_f$  0.31 (SiO<sub>2</sub> TLC, Benzene,

I-B). It gave a pink to blue to green colour with Libermann-Burchard reagent. The mass spectrum of the sterol exhibited fragment ions at  $m/e$  414 ( $M^+$ , 91%), 399 ( $M-CH_3$ , 43%), 396 ( $M-H_2O$ , 28%), 381 [ $M-(CH_3 + H_2O)$ , 15%], 273 ( $M-C_{10}H_{21}$ , 79%), 271 (74%), 255 ( $m/e$  273- $H_2O$ , 72%), 231 ( $m/e$  273- $C_{10}H_{19}$ , 48%) and 213 ( $m/e$  231- $H_2O$ , 49%), consistent with the fragmentation pattern which can be expected of  $\gamma$ -sitosterol<sup>8</sup>.

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#### PHYTOALEXIN SYNTHESIS IN RAGI LEAVES INFECTED WITH *PYRICULARIA SETARIAE* AS INFLUENCED BY PHENYLALANINE AND GLUCOSE

EVER since Müller and Börger<sup>1</sup> proposed the "phytoalexin concept" in host-parasite interaction, several reports appeared illustrating their role and occurrence in many plant diseases. In short, phytoalexins are a class of compounds synthesised by plants in response to pathogenic invasion. Recently<sup>2,3</sup>, it has been conceded that non-pathogens or even mechanical stimulus can induce phytoalexin production in plants. These compounds play a very vital role in the defence mechanism of plants particularly against infectious agents. In the present communication, the presence of phytoalexin has been observed in ragi (*Eleusine coracana* Gaertn.) plants infected with the blast pathogen *Pyricularia setariae*, Nishikado and the effect of phenylalanine

and glucose on the phytoalexin biosynthesis has been reported.

From two months old crop of ragi, healthy and diseased leaf material was collected and cut into bits of 10 cm. Twenty g of the material was floated in large petridishes containing (i) distilled water, (ii) 0.005 M glucose and (iii) 0.005 M DL phenylalanine and incubated for 24 hr at room temperature ( $28 \pm 1^\circ C$ ). They were swiftly rinsed in distilled water and extracted with 70% boiling methanol for 20 minutes. The remaining tissues were homogenized in a waring blender and re-extracted with hot methanol. The methanol fractions were pooled, volume condensed *in vacuo* and extracted with peroxide-free ether for 12 hr in the cold. The final ether fraction taken in a suitable aliquot of methanol<sup>4</sup> was tested for phytoalexin activity by observing on spore germination of *Helminthosporium oryzae* Breda de Haan and germ tube development of the organism<sup>5</sup>. Since many of the phytoalexins reported so far have got phenolic nuclei, the total phenol level in the extracts of leaves were estimated using Folin-Ciocalteu reagent<sup>6</sup>. The compounds were also separated on chromatograms (27  $\times$  23 cm Whatman No. 1 filter-paper) in a solvent system of *n*-butanol: acetic acid: water:: 4:1:1 (v/v). A 0.1% alkaline solution of diazotized sulphanilic acid<sup>7</sup> served as the detection reagent. The results are presented in Tables I and II.

TABLE I  
Total phenols in ragi leaves

Treatments	Total phenols* in $\mu g/g$ of fresh leaves
Healthy leaves suspended in distilled water	7.2
Infected leaves suspended in distilled water	8.3
Healthy leaves suspended in glucose	20.8
Infected leaves suspended in glucose	21.9
Healthy leaves suspended in phenylalanine	13.8
Infected leaves suspended in phenylalanine	23.2

\* Expressed in Catechol equivalents.

The blast infected leaf suspended in phenylalanine recorded the highest inhibitory activity towards *H. oryzae* spore germination and germ tube development. This was followed by the infected leaf suspended in glucose. Interestingly, the extract of

TABLE II  
Bioassay of phytoalexin from ragi leaves

Treatments	*Per cent germination	Length of germ tube (in $\mu$ )	Germination pattern
Healthy leaves in distilled water	.. 100.0	..	Profuse mycelial growth
Infected leaves in distilled water	.. 75.0	22.68	Malformations of germ tube, contorted growth
Healthy leaves in glucose	.. 75.0	111.24	Appressoria formed, beaded germ tube
Infected leaves in glucose	.. 44.0	11.07	Very short germ tubes with bulbous ends
Healthy leaves in phenylalanine	.. 50.0	29.70	Poor growth of germ tube, numerous appressoria formed, bulbous malformation
Infected leaves in phenylalanine	.. 10.0	7.02	Very poor growth of germ tube, no malformations
Control (Distilled water)	.. 100.0	..	Profuse and rapid growth

\* Observations taken after 12 hr of incubation.

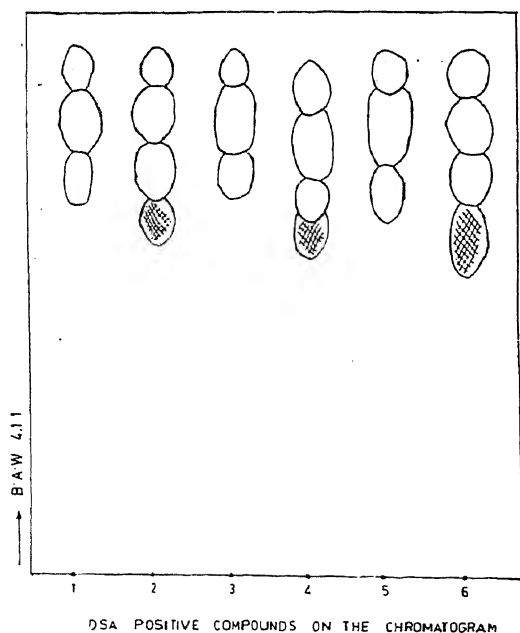


FIG. 1. 1. Healthy leaves suspended in distilled water. 2. Infected leaves suspended in distilled water. 3. Healthy leaves suspended in glucose. 4. Infected leaves suspended in glucose. 5. Healthy leaves suspended in phenylalanine. 6. Infected leaves suspended in phenylalanine.

healthy leaf with distilled water treatment had no inhibitory activity. The activity of phytoalexin paralleled with the total phenol levels in the tissues; thus the infected leaf fed with phenylalanine recorded higher quantities of phenols than others; healthy tissues possessed only the least amount. The chromatographic studies revealed that the extracts of the infected leaf contained an additional DSA positive spot (Fig. 1) which might probably be the phytoalexin. This is further confirmed by the fact that this compound was strikingly absent in healthy leaves.

Infected tissues suspended in phenylalanine recorded a high phytoalexin activity. Persuasive evidences are there<sup>8-10</sup> to indicate that aromatic amino acids participate in the synthesis of secondary metabolites in higher plants mediated by phenylalanine ammonia-lyase (PAL-ase) and tyrosine ammonia-lyase (TAL-ase). That there is three-fold increase of phenols in the infected tissues candidly justifies the accelerated synthesis of defence chemicals in response to infection<sup>11</sup>. The physico-chemical and biological properties of the phytoalexin needs further study. Because of the existing confusion in the terminology of phytoalexin, it is befitting here to recall the modified definition put forth by Kuc', "the term phytoalexin should be used in a broader sense to include all chemicals contributing to disease reaction"<sup>3</sup>.

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#### DEVELOPMENT OF BLACK ROT CAUSED BY *XANTHOMONAS CAMPESTRIS* IN ROOTED DETACHED CABBAGE LEAVES

BLACK rot is an important disease of cabbage and has been reported from different parts of the country<sup>1-5</sup>. Several cabbage lines have been found to be resistant and/or highly tolerant to the disease in India<sup>2</sup>. Often the quantity of seeds of several germ plasm is so small that it becomes insufficient for testing them against different isolates of the pathogen. Moreover, seed multiplication is a problem in the plains of India. In this note technique used in growing rooted detached leaves of cabbage along with pathogenicity tests on susceptible and highly tolerant lines on rooted detached leaves as well as on attached leaves is given.

Leaves were detached from cabbage plants grown in pots and were placed in nutrient solution contained in 250 ml Erlenmeyer flasks in the laboratory in such a way that the cut ends of the leaves were immersed about 1" in the solution. The nutrient solution [ $\text{KNO}_3$  1 g;  $\text{KH}_2\text{PO}_4$  1 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  1 g;  $\text{Ca}(\text{NO}_3)_2$  4 g; and  $\text{FeCl}_3$  solution 1 drop/litre] was diluted with distilled water (1 : 6) before being dispensed in flasks.

All the leaves, 16 each of the susceptible Pride of India and highly tolerant, Wis. 709-748 bc formed roots in the flasks and in course of time there was abundant root development (Figs. 1 and 2) in both the lines. The leaves remained healthy, turgid and green for more than 2 months in the laboratory. From time to time nutrient solution

was added to make good the loss due to evaporation.



FIG. 1. Root development in detached cabbage leaf in nutrient solution.

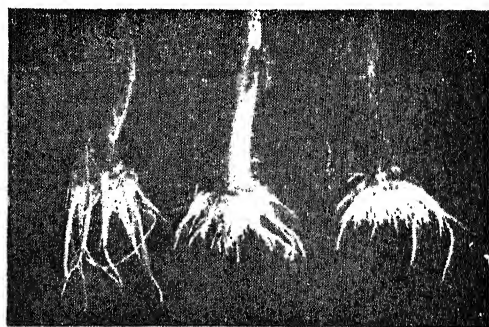


FIG. 2. Abundant root development in detached cabbage leaf.

The bacterial suspension from 48 hr old culture of *Xanthomonas campestris* originally isolated from cabbage was introduced (Ca.  $3 \times 10^7$  cells/ml) into the intercellular spaces of the cabbage leaves (Ca. 0.1 ml) in the field, in pots and in rooted detached leaves by hypodermic syringe (needle No. 24) using injection-infiltration technique of Klement<sup>3</sup>. There were four replications of each treatment consisting of four leaves and each leaf was inoculated at two places.

Initial necrosis was observed both in Pride of India (susceptible) and Wis. 709-748 bc (highly tolerant). In Pride of India there was much spread

of the disease from the site of inoculation whereas in the latter there was no such spread of the disease from the site of inoculation except in case of one leaf of a potted plant in a different experiment. The reaction of susceptible and highly tolerant lines was similar in field, pots and in rooted detached leaves. The experiment was repeated and results were same in the two tests. Similar results were obtained in apple scab in detached and attached leaves<sup>4</sup>.

The technique may be useful in testing pathogenicity of *X. campestris* with economy of space and seeds. Further work is in progress in this programme which is part of the All-India Co-ordinated Vegetable Improvement Project, I.C.A.R.

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#### A NEW RECORD OF PESTALOTIOPSIS VERSICOLOR (SPEG.) STEYART ON CITRUS FRUITS

VARIOUS fungi causing rot of *Citrus* fruits in the market have been reported from all over the world. *Alternaria citri*<sup>1</sup>, *Bartlinia robillardoides*<sup>2</sup>, *Beltrania rhombica*<sup>3</sup>, *Coniella citri*<sup>3</sup>, and *Glomerella cingulata*<sup>2</sup> have been reported causing rot of *Citrus* fruits from Jabalpur. During December 1972 and January 1973 fruits of *Citrus aurantium* with some new type of rot were noticed and collected from the local market. Young spots were small dark brown and mature ones ash coloured with black dots in the central region. The pathogen was isolated and axenic culture obtained. On identification it was found to be *Pestalotiopsis versicolor* (Speg.) Steyart.

The acervuli are dark coloured and the conidia are borne on small conidiophores. Conidia are 3 to 5 septate, mostly 4 septate central cells dark coloured and end cells hyaline. Three cilia

are present at the apical end. Conidia measure  $15-25 \times 3.3-5 \mu$ , average  $20 \times 4.5 \mu$ .

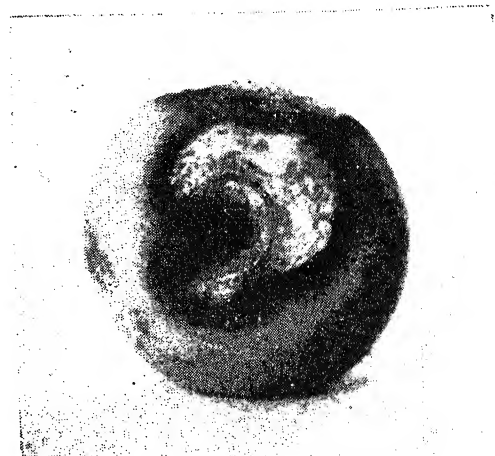


FIG. 1. *Citrus sinensis* showing rotting due to *Pestalotiopsis versicolor* at 30° C.

Pathogenicity of *P. versicolor* on *Citrus aurantium* fruits was established successfully. It was found to be a wound parasite. Artificial inoculum consisted of mass of mycelium mixed with spores. Fruits were wounded by pricking with needle and inoculum kept on it. Moist cotton pads were also kept over the inoculated region. The infection appeared within 5 to 7 days. Besides *C. aurantium*, *C. sinensis* and *C. medica* var. *acida* were also tested. The former gave positive while the latter gave negative response.

Effect of temperature on the spread of rot was also investigated. Maximum rotting of fruits was noticed at 30° C in a range of 15° to 35° C.

Review of literature shows that so far there is no record of *P. versicolor* on any *Citrus* fruits. The present note is the first record of the same from Jabalpur (India). The culture of the pathogen has been deposited in C.M.I., Kew, England, I.M.I. No. 172974.

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# ALKALINE PHOSPHATASE ACTIVITY IN THE OVARY OF THE CAT-FISH, *CLARIAS BATRACHUS* (LINN.) DURING MATURATION

THOUGH several histochemical and biochemical reports have appeared in the past on the phosphatase activity during the embryonic development of various animals,<sup>1-5</sup> such studies on differentiating tissues, like the ovary of fish, have relatively been few.<sup>6-7</sup> The present communication describes the quantitative changes in the activity of a well-known, non-specific phosphomonoesterase, alkaline phosphatase (Orthophosphoric monoester phosphohydrolase, EC No. 3.1.3.1), in the ovary during its growth and maturation in *Clarias batrachus* (Linn.).

Live specimens of *C. batrachus* were collected from local ponds during different seasons of the year. The fishes were dissected out and their maturity stages arbitrarily established on the basis of shape, colour, size and gonad weight. The ovaries were classified into four distinct stages, viz., recovering (stage I), maturing (stage II), mature (stage III) and spent (stage IV). The ovaries falling into these stages were removed and blotted on to a filter-paper so as to remove any adhering fluid. A weighed amount of tissue (0.1 g) was then taken, homogenized in 5 ml of ice-cold distilled water and centrifuged for 5 minutes at 3,000 r.p.m. 1 ml aliquot of the supernatant was assayed for the enzyme activity. Method of enzyme estimation was the same as described elsewhere<sup>8</sup>. For each maturity stage triplicate determinations were made and the mean enzyme activity plotted in Fig. 1.

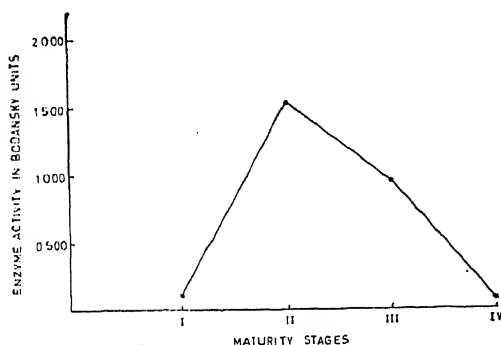


FIG. 1

As would be evident from Fig. 1, the ovarian tissue exhibited marked alkaline phosphatase activity during its development. The level of enzyme activity, however, registered marked variations from one maturity stage to another. The spent ovary (stage IV) was characterized by very low alkaline phosphatase activity. A distinct increase in the enzyme activity accompanied the earlier stages of

ovarian growth, the highest value being obtained in the maturing ovaries (stage II). A fall in the alkaline phosphatase activity occurred during the mature stage (stage III) when the ovary was full of fully grown oocytes. The ovary during the recovering phase (stage I) started accumulating this enzyme again. The rise and fall in the alkaline phosphatase activity observed during maturation seem to conform well with the findings on other fishes<sup>6,7</sup>.

The above observations indicate that the activity of alkaline phosphatase in the ovary of *C. batrachus* varies with the stage of its differentiation. The ovary of the fish undergoes marked cytomorphological changes during its maturation and growth. During the maturing stages (stage II) the oocytes grow rapidly, presumably by the active synthesis and accumulation of reserves, mostly protein. It is interesting to observe that the ovary during this synthetic phase records the highest alkaline phosphatase activity, indicating the possible involvement of this enzyme in protein synthesis. Bradfield<sup>9</sup> has earlier emphasized that phosphatases may be a part of the enzyme system involved in the liberation of the newly synthesized protein from a complex with the nucleic acid. A drop in the activity of alkaline phosphatase observed in the mature ovary with fully grown oocytes points towards a fall in the synthesis of reserves, as the oocytes by this stage appear to complete their growth and differentiation. The lowest level of enzyme activity during the spent phase signifies that the enzyme apparently plays no significant physiological role during this stage.

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### CHROMOSOMES OF *PHIDIPPUS* SP. (SALTICIDAE, ARANEAE)

CHROMOSOMAL data are available on as many as 38 species of spiders covering 15 genera of the family Salticidae<sup>1-7</sup>. The object of this short communication is to report on the chromosomes in a species belonging to the genus *Phidippus* which has so far not been worked out cytologically.

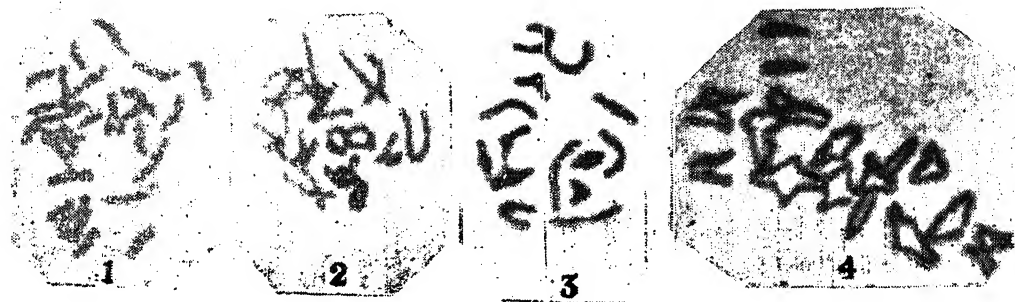
The diploid complement, as seen at spermatogonial mitoses, consists of twenty-eight acrocentric elements. All of them are rod-shaped with one of the ends nearly pointed, the other rounded. Twenty-six of them are autosomes and the remaining two are two sex chromosomes although the latter are not distinguishable from the autosomes at this stage (Fig. 1). In the early diakineti-

cal stage in the geological history, their sex-chromosome mechanism must necessarily be of great antiquity—unlike most other 'multiple mechanisms' which may be of rather recent origin.

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FIGS. 1-4. *Phidippus* sp. Fig. 1. Spermatogonial metaphase. Fig. 2. Early diakinesis. Fig. 3. Late diakinesis. Fig. 4. Anaphase I.

cal stage of meiosis, however, the two sex chromosomes, differentiable from the autosomal bivalents by their prominent heteropycnosis, associate themselves closely to form a single mass (Fig. 2). Two of the autosomal bivalents are ring-shaped whereas the rest have a single chiasma each (Fig. 2). Fourteen chromosomal elements are thus observed during late diakinesis, the sex chromosome pair being recognizable from the autosomal bivalents as two univalent elements always lying close together (Fig. 3). Metaphase I is normal with the thirteen autosomal bivalents oriented on the equator and the two sex chromosomes move precociously to one of the poles of the spindle (Fig. 4).

Thus, except for *Myrmarachne formicaria* (Hackman, 1948), which has  $2n=23$  in the male ( $n=12$  and an XO-system of sex-determining mechanism), all other male members of this family studied to date have  $2n=28$  ( $n=15$  and an  $X_1X_2O$ -system of sex-determining mechanism<sup>2-3,6</sup>). Previous studies<sup>1-7</sup> indicate that most male spiders possess an  $X_1X_2O$  sex-chromosome constitution while the female has an  $X_1X_1X_2X_3$  constitution. Inasmuch as the spiders extend back to the

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### OCCURRENCE OF A COMMENSAL HYDROID *EUGYMNANTHEA* SP. IN A MARINE FOULING MOLLUSC *CONGERIA SALLEI* RECLUZ (PELECYPODA)

COMMENSAL associations between Cnidaria and many marine bivalves have been known for quite some time. A common hydroid recorded from bivalve molluscs is a genus *Eugymnanthea* which is represented so far by four species, living exclusively in the mantle cavity of a wide range of hosts<sup>1,2</sup>. Of the four species recorded so far *E. inquilina* and *E. polinanti* were obtained from Italian waters<sup>3,4</sup>. The remaining species of *Eugymnanthea*, viz., *E. ostrearum* and *E. japonica* were recorded respectively from Puerto Rico and Japan<sup>5,6</sup>. In India, *Eugymnanthea* sp. has recently been reported from the mantle cavity of both tereid and pholadid

wood borers<sup>7</sup>. However, occurrence of the same from a fouling organism in Indian waters has not been reported so far.

*Congeris sallei*, a bivalve mollusc, has of late attracted the attention of biologists studying the problem of marine fouling in Visakhapatnam harbour. During the course of studies on *C. sallei*, several specimens of the animal were found to be infested with the hydroids of the genus *Eugymnanthea*. As many as 800 simple or branched polyps were found to inhabit one single animal. The hydroid was normally present on the gills. The salient features of the hydroid encountered in the present study along with the various dimensions are described below :

The round basal disc of the hydroid is deeply embedded in the gill tissue and the polyps are athenate. It may be observed from Fig. 1 that both hydrorhizae and gonothecae are absent and the polyps have a single whorl of tentacles. The gonophores are produced near the base of the polyps.



FIG. 1. Colony of *Eugymnanthea* sp., showing basal disc and gonophores.

Length of polyp ..	200-1080 μ
Diameter of polyp at the distal end ..	80-280 μ
Diameter of polyp near hypostome ..	80-160 μ
Diameter of basal disc ..	120-320 μ
Number of tentacles in a whorl ..	8-20
Length of tentacles ..	20-80 μ
Length of gonophore ..	80-800 μ
Diameter of gonophore ..	60-480 μ

The medusa stage of *Eugymnanthea* sp., however, could not be observed in the present studies. Since the specific identification of the organism primarily depends on the characters of both the polyp and medusa stages, in the absence of medusae, the species could not be established. The polyps, however, show characteristics similar to those described from woodborers in Cochin waters<sup>7</sup>.

The hydroid *Eugymnanthea* sp. apparently establishes an intimate association with the host. Although the hydroid is deeply embedded in the gills of the host, it does not appear to affect the tissues of *C. sallei*, as was evident from the healthy nature of the infected animals. Thus there is no indication of parasitism and the association between *Eugymnanthea* sp. and *C. sallei* may be treated as commensalic in nature. This is further supported by the observations of Mattox and Crowell<sup>5</sup> that absence of perisac and stolon and the presence of a round basal disc in *Eugymnanthea* may be considered as adaptive features of commensalism.

The authors wish to thank Shri S. V. S. Rao, Officer-in-Charge of this laboratory, for his constant encouragement during the course of the present study. The authors wish to thank Shri S. S. Ganti of this laboratory for his suggestions and critical evaluation of the manuscript.

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#### CYTOLOGICAL EFFECTS OF 2,3-DIHYDRO-2,2-DIMETHYL-7-BENZOFURANYL METHYL-CARBAMATE "CARBOFURAN"

FURADAN (Common name Carbofuran) is a systemic insecticide used in agriculture which provides excellent control of insect pests affecting rice and other plants of economic importance. It is becoming increasingly evident that many of the commonly used pesticides have radiomimetic properties. Some pesticides have clearly been shown to be mutagens and can induce gene mutations<sup>1</sup>. Thus pesticides are agents which are potential for promoting evolution<sup>2</sup>. Some of the results from a study undertaken to determine the effects of Furadan on *Allium cepa* chromosomes are reported here.

Germinating bulbs of *Allium cepa* with their roots were immersed for 2 and 4 hours at concentration of 0.1% Furadan. Its solubility in



water is 250–700 ppm at 25° C. All the experiments were carried out at room temperature. After the designated period of treatment the immersed roots of each bulb were thoroughly washed in tap-water and fixed in freshly prepared acetic alcohol (1:3) and stained by the Haematoxylin squash technique<sup>3</sup>.

The percentages of the mitotic stages were, however, the same as in the control experiment. At the metaphase stage the chromosomes were irregularly scattered in the cell. The prophase-metaphase type was common (Fig. 3). The

induced prophase-metaphases may be attributed to the inhibition of the spindle formation. The chromosomes would, thus, remain nearly in their arrangement as they were during the prophase stage. Abnormalities such as fragments (Figs. 1 and 2), clumping, scattered anaphases were also observed in two time intervals. Other cytological effect which is more pronounced was the occurrence of chromosome contraction. This is not very surprising since other carbamates have been known to bring about chromosome contraction<sup>4,5</sup>. A 4-hour treatment induced differential contraction in the metaphase chromosomes (Figs. 5 to 7). The observed difference in contraction was not considered to be a result of a difference in the stage of mitosis. The frequency of cells with chromosome aberrations induced by Furadan failed to show any clear relationship with regard to duration of treatment.

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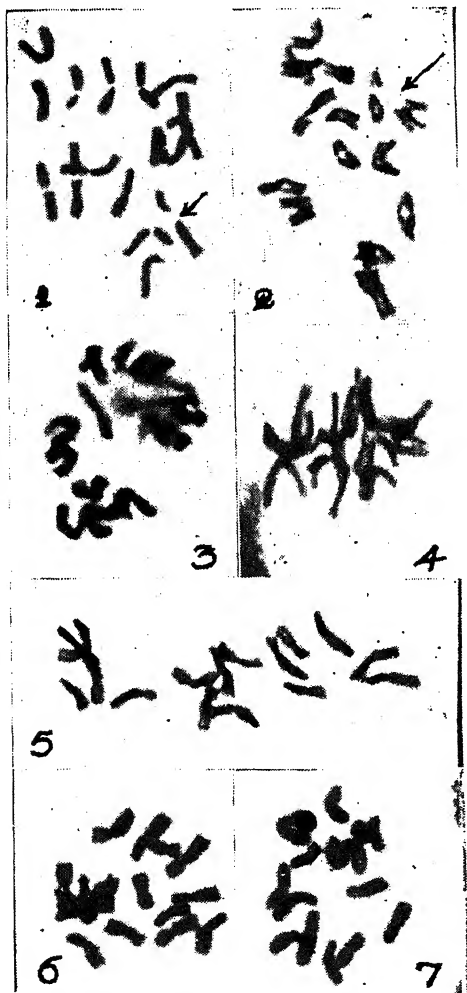
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#### INFLUENCE OF FERTILIZERS ON THE DYE-BINDING PROPERTIES OF MULBERRY LEAF PROTEIN

IN an earlier paper Gupta *et al.*<sup>1</sup> presented the general dye-binding pattern of mulberry leaf protein. This paper presents the results of an investigation particularly undertaken to determine the influence of nitrogenous fertilizers on dye-binding nature and properties of mulberry leaf protein.

Local mulberry (*Morus alba* Linn.) plants were grown at Central Sericultural Research Station, Berhampore (West Bengal), in two sets of experimental plots. One set was treated with ammonium sulphate @ 300 kg, 600 kg, and 900 kg, of nitrogen per hectare as soil application and the other with urea @ 50 kg and 100 kg of nitrogen per hectare as foliar application. Randomised leaf samples were collected and mulberry leaf protein



FIGS. 1-7. Chromosome aberrations in root tip cell of *Allium cepa* following 2 and 4 hour treatment with Furadan. Figs. 1-3. 2 hr. Figs. 5-7 4 hr. Figs. 1-2. Metaphase chromosome breakage,  $\times$  ca, 1,300. Fig. 3. Prophase-Metaphase,  $\times$  ca, 1,300. Fig. 4. Metaphase Control.  $\times$  ca, 1,300. Figs. 5-7. Metaphase chromosomes exhibiting different degrees of contraction,  $\times$  ca, 1,300.

TABLE I

	Ammonium sulphate as soil application	Urea as foliar application	General
Correlation coefficient ( <i>r</i> )	0.9857	0.9599	0.9717
Regression coefficient ( <i>b</i> )	3.1705	2.7544	2.9759
Constant ( <i>a</i> )	-10.0873	-3.7769	-7.4392
Regression equation ( <i>y</i> )	$-10.0873 + 3.1705x$	$-3.7769 + 2.7544x$	$-7.4392 + 2.9759x$
Standard error of regression coefficient ( <i>sb</i> )	0.1947	0.2322	0.1507
F-value for testing significance of regression	265*	140*	389*

estimated by Kjeldahl method as well as Ion-binding method.

It has been observed that the protein percentage in leaf samples as estimated by Kjeldahl method increased from 14.21 to 22.25% and DBC (Dye-binding capacity) values from 36.0 to 60.0% when ammonium sulphate was used as soil application. Again the protein percentage increased from 14.43 to 20.75% and DBC values from 36.0 to 57.4% when urea was used as foliar application. In both the cases the amount of crude protein in leaf increased as levels of nitrogen application increased.

In order to understand whether nature and mode of application of fertilizer produced any influence on the dye-binding capacity value of mulberry leaf protein or on the physico-chemical properties of mulberry leaf protein, replicated leaf samples of ammonium sulphate and urea treated plots were considered separately for regression study along with mixed samples for general regression analysis.

In the regression analysis significance of regression coefficient was first considered which lead to the partitioning of the total variation into variation due to regression and variation due to deviation from regression and to calculate  $F = MR/MD$  with 1 and  $n - 2$  degrees of freedom where MR has been the mean sum of squares due to regression and MD the mean sum of squares due to deviation from regression. The results of the statistical analysis are given in Table I.

The equality of first two regression lines was tested by calculating

$$F = \frac{(SS2 - SS1)/2}{(SS1(n1 + n2 - 4))}$$

with 2 and  $n1 + n2 - 4$  degrees of freedom SS1 being the sum of squares due to deviation from common regression. The F-value was found as 1.053 which was not significant at 5% value. Thus, first

two regression equations were the same and the common regression equation could also be utilised by combining the data from the series of fertilizer treated samples.

Otherwise, it could be concluded that instead of screening the samples and computing regression equations for different fertilizer applications the common regression, equation  $y = -7.4392 + 2.9759x$  where *y* is the DBC value and *x* is the protein percentage could serve the purpose of estimating mulberry leaf protein. Dye-binding capacity (DBC) values corresponding to the protein percentage of different leaf samples are presented in Fig. 1. The figure shows a fair scattering of points.

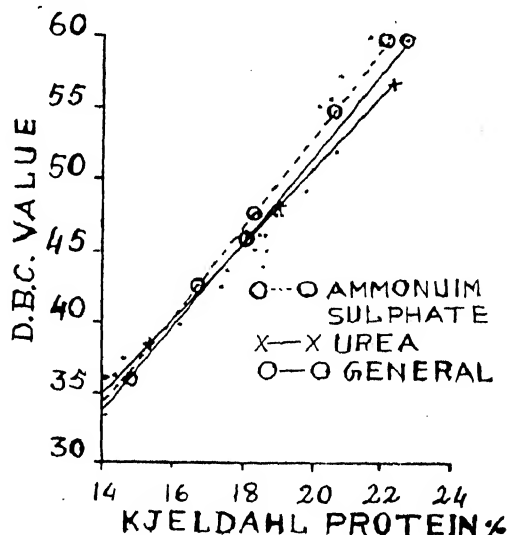


Fig. 1

The reason for such scattering may be due to the fact that water soluble nitrogenous compounds may react with the dye, and remain in solution which

are not estimated by this method as described by Outen *et al.*<sup>2</sup>. The dye-binding properties of mulberry leaf protein does not change with the nature and mode of application of nitrogenous fertilizers and at the same time scattering of points have no relation with these two factors.

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## EMBRYOLOGICAL STUDIES IN COMPOSITAE

### V. On the Embryology of *Eclipta alba*, Hassk.

RECENT Contributions to the embryology of Compositae include those of Davis (1968), Deshpande (1964), Maheshwari Devi (1953) and Sundara Rajan (1972, 1973, in press)<sup>1-2,4-5</sup>.

The present investigation deals with some aspects of embryology of *Eclipta alba*, Hassk. This plant belongs to the tribe *Heliantheae*, sub-tribe *Verbesineae*.

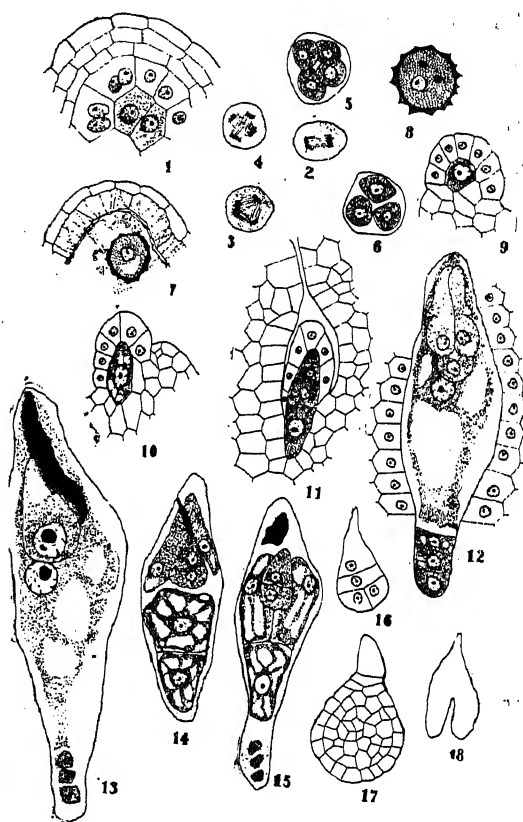
The observations presented are based on the material collected around Bangalore and fixed in F.A.A.

A transection of a young anther lobe shows an epidermis, an endothecium, a middle layer, a tapetum and two microspore mother cells (Fig. 1). In the presence of two linear rows of microspore mother cells in the microsporangium, *Eclipta alba* is similar to *Spilanthes acmilla* and *Blainvillea rhomboidea* of the sub-tribe *Verbesineae*. The tapetal cells are uninucleate in the beginning but later become binucleate. Tapetal mitotic activity synchronises with the meiotic activity of the microspore mother cells.

The microspore mother cells undergo the usual reduction divisions (Figs. 2, 3 and 4) to produce decussate and tetrahedral tetrads of microspores (Figs. 5 and 6). At the time when the microspores are separating apart from the tetrad, the walls of tapetal cells break down and the protoplasts fuse and surround the microspores (Fig. 7). Consequently the tapetum is of the plasmodial type. In this respect *Eclipta alba* is not only in agreement

with the other members of the sub-tribe *Verbesineae* but also agrees with the majority of Compositae also.

The mature pollen grain is shed at the 3-celled stage (Fig. 8) as in other Compositae. It has an echinate exine and a thin intine. The endothecium exhibits prominent fibrillar thickenings at the time of anther dehiscence and the epidermis remains over as a prominent layer (Fig. 7).



FIGS. 1-18. Fig. 1. Transection of an anther lobe,  $\times 2,000$ . Figs. 2-4. Meiotic stages in microspore mother cells,  $\times 2,000$ . Figs. 5-6. Decussate and tetrahedral tetrads of microspores,  $\times 2,000$ . Fig. 7. T.S. of anther showing plasmodial tapetum and fibrillar endothecium,  $\times 900$ . Figs. 9-10. Archesporium and megaspore mother cell respectively,  $\times 2,000$ . Fig. 11. Linear tetrad of megaspores,  $\times 2,000$ . Fig. 12. Organised embryo sac,  $\times 2,000$ . Fig. 13. Embryo sac showing fertilisation,  $\times 2,000$ . Figs. 14-15. Early divisions of zygote and endosperm,  $\times 900$ . Figs. 16-18. Embryogeny. (Fig. 16,  $\times 2,000$ ; Fig. 17,  $\times 900$ ; Fig. 18,  $\times 200$ .)

The ovary is inferior, bicarpellary and unilocular having a single unitegmic tenuinucellate ovule on basal placenta. The ovule arises as an erect hump of cells and later assumes the usual anatropous condition. When the ovular primordium is still

erect, a single hypodermal archesporial cell becomes differentiated (Fig. 9) and it directly functions as the megaspore mother cell (Fig. 10). Meiosis in the megaspore mother cell is initiated after the ovule attains the anatropous condition. A linear tetrad of megaspores results after meiosis (Fig. 11) in which the chalazal spore is functional. The nucleus of the functional megaspore undergoes three successive free nuclear divisions contributing to the formation of an eight-nucleate embryo sac of the Polygonum type. The organised embryo sac is seven-celled (Fig. 12) with three superposed uni-nucleate antipodal cells completely occupying the narrow chalazal region of the embryo sac. The egg apparatus consists of two long prominently beaked synergids and a median egg (Fig. 12). The two polar nuclei fuse before fertilisation and the secondary nucleus lies closely appressed to the egg.

Fertilisation is porogamous. Triple fusion is followed by syngamy (Fig. 13). Generally the entry of the pollen tube crushes the synergids, but sometimes the pollen tube enters the embryo sac in between the two synergids resulting in their persistence even after the first division of the zygote and the primary endosperm nucleus.

The primary endosperm nucleus divides almost simultaneously with the zygote. The first division of the primary endosperm nucleus is transverse and is followed by a wall (Fig. 14). Consequently the endosperm is of the *ab initio* cellular type. Cellular endosperm seems to be a characteristic feature of the sub-tribe *Verbesineae* as in the other two investigated members, *Blainvillea rhomboidea* and *Spilanthes acmilla*<sup>5</sup>. Second division of the endosperm takes place early in the micropylar cell and it is vertical (Fig. 15).

The antipodal cells generally degenerate after the initial development of the endosperm is complete.

The first division of the zygote is transverse resulting in the formation of a 2-celled proembryo. The terminal cell undergoes a vertical division and the basal cell divides transversely to form a 'T'-shaped proembryonal tetrad (Fig. 16). The further development of the embryo conforms to the Asterad type<sup>3</sup>. The suspensor is short and is made up of only one cell (Fig. 17). The mature embryo is dicotyledonous (Fig. 18).

My thanks are due to the Principal and Head of the Department of Botany, St. Joseph's College, for the facilities.

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#### PERITHECIAL STAGE OF *CYLINDROCLADIUM ILICICOLA* BOED. AND REIT.

DURING October-November, the author observed severe leaf spot disease of *Eucalyptus globosus* Linn. growing at the local gardens. The disease made its appearance from margin or apex and proceeded towards midrib and base respectively. The spots were greyish-brown in colour and demarcated by yellow halo. Defoliation is also common. Isolations from such spots yielded the richly sporulating culture of *Cylindrocladium ilicicola* (Hawley) Boed. and Reit. From perusal of the literature it revealed that neither conidial nor perithecial stage is reported from India.

The colonies are reddish-brown with abundant aerial mycelium; hyphae subhyaline, septate, branched,  $2.5-3.0\ \mu$  wide; chlamydospores are most frequent and sometimes aggregating to form sclerotia-like bodies, both intercalary or terminal, conidiophores penicillate with varying length,  $4.3-7.2\ \mu$  in width at the base, dichotomously branched near the apex. Primary branches  $24.5-32.7 \times 3.0-4.7\ \mu$ . Secondary branches  $12.5-18.2 \times 2.0-3.5\ \mu$ , ultimate branches bearing 2-4 phialides which are  $10.2-13.4 \times 2.5-3.0\ \mu$  (Fig. 1), the main axis ending in subspherical to

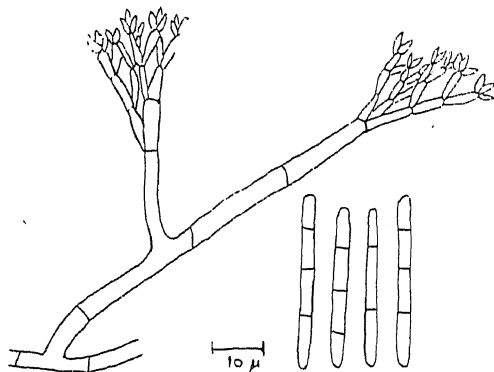


FIG. 1. Camera lucida drawings of conidiophore and conidia of *Cylindrocladium ilicicola*.

clubshaped papilla; conidia cylindrical, 2-3 septate (mostly 3-septate), matured conidia becoming

vacuolate, hyaline,  $63.0 \times 3.6$  ( $57.6-64.8 \times 2.7-4.3 \mu$ ).

After about a month orange-yellow coloured perithecia of an ascomycetous fungus was developed which on examination revealed as *Calonectria ilicicola* Boed. and Reit.

Perithecia scattered, subglobose to oval, orange-yellow, pseudoparenchymatous wall, distinct papilla with ostiole (Fig. 2)  $307.4-214.6$  ( $261-522 \times 232-319 \mu$ ); asci clavate with long stipe, hyaline, unitunicate, 4-8 ascospores (Fig. 3) which are

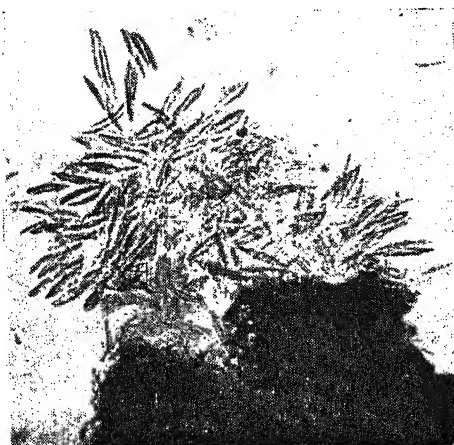


FIG. 2. Showing the photomicrograph of perithecium of *Calonectria ilicicola* ( $\times 440$ ).



FIG. 3. Photomicrograph showing the asci and ascospores of *C. ilicicola* ( $\times 1,200$ ).

cozed out by the rupture of the apical portion, paraphyses absent,  $68.4 \times 14.4$  ( $57.6-79.2 \times 10.8-18.0 \mu$ ); ascospores fusoid to falcate, curved, mostly 3-septate, hyaline, becoming highly vacuolate on maturity,  $59.0 \times 5.2$  ( $46.8-75.6 \times 3.9-7.2 \mu$ ).

In order to establish its relation with conidial stage the monoconidial cultures were raised and in each case the perithecia resulted in about a month. Simultaneously the single perithecial cultures were also raised which resulted both the conidial and perithecial stage.

So far the perithecial stage of five species of *Cylindrocladium*<sup>1-5</sup> have been discovered and all of which belong to genus *Calonectria*.

The author is grateful to Prof. Jafar Nizam for his kind encouragement and facilities.

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September 17, 1973.

S. M. REDDY.

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#### PRELIMINARY STUDIES ON THE EFFECT OF CERTAIN CHEMICALS, INTERFERING WITH PROTEIN AND NUCLEIC ACID SYNTHESIS, ON THE PROCESS OF VERNALIZATION

IT is now well established that photo-induction of flowering is intimately related to synthesis of nucleic acids in the apical meristem<sup>1</sup>. As far as vernalization is concerned, studies are few and results inconclusive. In the present investigation, effect of actinomycin-D, chloramphenicol, chromomycin A-3 and porfirimycin has been studied on the process of seed vernalization of (i) *Brassica campestris* L., Yellow Sarson T. 42, (ii) *Cicer arietinum* L., Ujjain 2 and (iii) *Eruca sativa* L., Culture 6518. Chloramphenicol disrupts protein synthesis at the polypeptide stage, while the rest interferes with the synthesis of nucleic acids at one stage or the other.

Seeds were soaked in aqueous solutions of various concentrations of the chemicals, for 6 to 8 hours, at room temperature and vernalized according to the technique described elsewhere<sup>2</sup>, the period of chilling being 2 weeks for *Brassica* and *Eruca* and 3 for *Cicer*. Two types of controls, (i) chilled after being soaked in water and (ii) normal, have been used for sowing along with the chemically treated ones. A final stand of 4 plants per pot was allowed. Time taken for anthesis by 10 individuals of each treatment, flowering in a serial order, was recorded, averaged and subjected to analysis of variance.

TABLE I

Effect of certain chemicals on the acquirement of vernalization status in *Brassica campestris* L.,  
*Cicer arietinum* L. and *Eruca sativa* L.

Chemical	Conc. ppm	Brassica		Cicer		Eruca	
		Anthesis in days	Delay over vernalized	Anthesis in days	Delay over vernalized	Anthesis in days	Delay over vernalized
Actinomycin D	1	38.4	0.4	63.7	0.6	45.1	2.7*
"	5	38.5	0.3	62.9	1.4	45.4	3.0*
Chloramphenicol	1	37.6	1.2	62.2	2.1	44.9	2.5**
"	10	41.0	2.2	63.6	0.7	48.3	5.9*
"	100	55.7	16.9*	70.4	6.1*	53.6	11.2*
Chromomycin A-3	1	37.6	1.2	62.5	1.8	43.9	1.5
"	10	41.0	2.2	62.0	2.3	45.1	2.7**
Porfirimycin	1	39.0	0.2	64.4	0.1	46.4	4.0*
"	10	47.8	9.0*	69.8	5.5*	50.6	8.2*
Control		46.6	7.8*	82.2	17.9*	55.8	13.4*
Water vernalized		38.8		64.3		42.4	
C.D. at 1% level		3.3		3.8		2.8	
C.D. at 5% level		2.4		2.8		2.1	

\* Significant at 1% level.

\*\* Significant at 5% level.

Data have been presented in Table I, from which it would be seen that in *Eruca* with the exception of 1 ppm chromomycin A-3, in the rest of the treatments there has been significant nullification of the chilling effect. In the other two plants, it is so with 100 ppm chloramphenicol and 10 ppm porfirimycin only. Higher degree of nullification of the chilling effect in *Eruca* might be due to its mucilaginous seed coat, which ensures a supply of the chemical to the embryo over a prolonged period.

Results reported above suggest a positive correlation between protein and nucleic acid synthesis on the one hand and acquirement of vernalization status on the other and are in conformity with those of De'vay<sup>3</sup>.

Further work is in progress.

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and

Dept. of Botany, B. POORNA SRI DEVI,  
Saugar University, Sagar, November 3, 1973.

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#### OCCURRENCE OF CERATOPHORUM HELICOSPORUM (SACC.) SACC. IN INDIA

EVERY year, during rainy season, a dematiaceous fungus has been observed on the foliage of chestnut trees. The fungus is entirely superficial and the mycelium often covers the entire under-

surface of the leaves, forming a smoky coating. The hyphae are hyaline, septate, branched and 2-3  $\mu$  in diameter, producing numerous sub sessile or shortly stipitate, dark brown conidia. The conidia are fusiform or cylindrical-clavate, tapering and curved at apex, truncate at base, with a prominent basal scar, 12-16 septate and 108-189  $\times$  12-14  $\mu$  in size (Fig. 1).

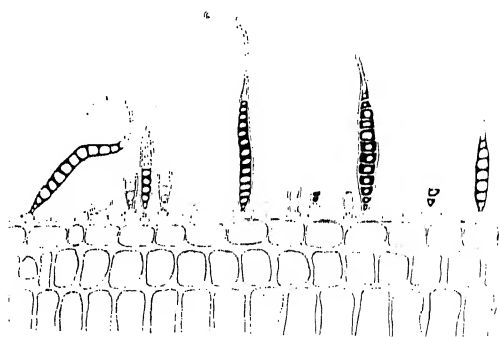


FIG. 1. *Ceratophorum helicosporum* on chestnut leaf.  $\times$  150.

The fungus belongs to the genus *Ceratophorum* Sacc. Several species are known to occur on diverse hosts, including those belonging to the family *Fagaceae*. Saccardo (1886) has recorded the occurrence of *C. epiphyllum* (B. and C.) Sacc. on leaves of chestnut. Hughes (1951) has indicated that this is identical with *C. helicosporum* (Sacc.) Sacc. The fungus described herein agrees with *C. helicosporum* very closely in shape, size and septation of the conidia and is, therefore,

considered as identical. The fungus has, so far, not been recorded from India. The fungus does not appear to cause any serious damage except that the leaves become unsightly.

Specimens of the fungus on chestnut leaves have been deposited in the Herb. Crypt. Ind. Orient., Indian Agricultural Research Institute, New Delhi.

The author is grateful to Dr. S. K. Bose for his help and guidance during the course of this investigation.

Government Hill Fruit

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Chaubattia (Almora), September 22, 1973.

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#### A SIMPLE TECHNIQUE FOR SPORE GERMINATION STUDIES

For the study of spore germination it is usual to place the solution on a clean microslide, add the spores to it and leave them in a moist chamber<sup>3</sup>. Non wettable spores such as those of Smuts, *Aspergillus* spp., *Penicillium* spp., *Lycoperdon* spp., float on the water drops and the germination results are inconsistent. Microscopic observation, staining, preparation of permanent slides are also difficult. In connection with our studies on spore germination in *Aspergillus flavus* we felt these difficulties and devised the following simple technique.

Dry spores of the fungus were smeared on the surface of a clean cellophane strip (18 × 24 mm) with the help of brush or a glass rod. A piece of Whatman filter-paper (18 × 30 mm.) is placed on a slide, 6 mm of one of its end projecting from the edge of the slide. The filter-paper is moistened with the required solution and the cellophane paper with the spores is spread over its surface. The microslide is kept over a support inside the petridish and the projecting filter-paper end is dipped into the solution in the petridish or a small cup. The lid of the petridish is lined with a moist filter-paper to keep up the high humidity and where the solution is kept in a cup another layer in the bottom of the dish. A line drawing of the assembly is shown in Fig. 1.

After the required period of incubation the filter-paper is removed from under the cellophane strip by holding the latter with a forceps. The cellophane strip with spores is mounted between the slide and

a cover glass using either lactophenol or the mountant with stain (cotton blue). Where the spores have to be processed for staining and preparing permanent mounts a strip of Scotch Magic Tape<sup>2</sup> may be used instead of cellophane. In that case the spores are brushed on the sticky side of the tape.

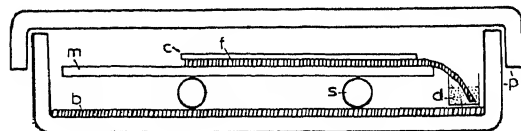


FIG. 1. Spore germination assembly (diagrammatic). c, cellophane; f, filter-paper; m, microslide; s, support; d, dish with solution; b, blotting paper; p, petridish.

This technique is found to give consistent results as compared with the conventional method. The method could also be used for studying the viability of the spore catches obtained on tapes such as Hirst trap (Burkard model)<sup>1</sup>. Rotary drum spore trap<sup>2</sup> and germination of spores in root exudates, chemical solutions, etc. This method has been found to be useful in testing the aerosol viability of *A. flavus* spores exposed on silk threads, in our experimental studies.

Care should be taken while mounting the cellophane on the moist filter-paper as the former tends to roll up, but becoming flat immediately on absorption of moisture. The cellophane could be held by two needles until it becomes flat. The cellophane or the Scotch tape are made of cellulose and are permeable to water. They act as a good substrate for the germination of spores. The spores are kept constantly on a moist bed without either sinking into water or drying up as in the conventional method. The set up also simulates some conditions as they occur in nature. This method could be followed by those working on the germination of spores.

One of us (E. R.) is thankful to the C.S.I.R. for the award of a Junior Fellowship.

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E. RATI.

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# CHIMERA IN *SANTALUM ALBUM* L.\*

FREAKS of nature amongst the flowering plants have been reported from time to time. Recently one such case has come to notice in the experimental forest of the Forest Research Laboratory, Bangalore, with sandalwood tree, *Santalum album*, the source of the well-known sandalwood oil, which is extensively used in soaps, perfumes and cosmetics. Normally, *Santalum album* is a perennial tree with profuse dark green foliage, requiring a few decades to yield the essential heartwood from which the renowned oil is extracted. But a 12-15-year old sapling has been noticed to distinctly vary from the rest in having, at about 6 feet height from the ground, two to three small twigs in which the entire foliage is having a yellow margin and green patches all along the midrib in contrast to the dark green foliage of the rest of the branches of the canopy (Fig. 1). But for this major deviation, this plant would have been a perfectly normal one like the others.



FIG. 1

The apparent variation was first suspected to be due to some kind of disease infection, particularly of virus or mycoplasma. So the twigs were treated with benlate<sup>1</sup> and ledermycin<sup>2</sup>, which were shown to have curative effect on the formidable sandal spike disease, now known to be caused by mycoplasma. But there was no visible improvement.

Neither had there been any change in the foliage colour of the fresh growth which came up after a deliberate pruning of the old twig. Further, such a change in the foliage colour was very much localised to those particular twigs. All this indicated the possibility of the cause being more deep-rooted than suspected. It is quite possible that it might very well be genic in nature.

Cases of obvious dimorphism in the manifestation of a character in any one given individual, either induced or spontaneous, are commonly referred to as chimeras and this subject has been discussed in detail in a review by Crammer<sup>3</sup> and more recently by Neilson-Jones in a monograph entitled *Plant Chimeras*<sup>4</sup>. Nowhere in this review nor in the subsequent related literature has there been any evidence of chimeras being observed in this species. It is, therefore, a new record and hence the scientific credulity.

How chimeras occur is so very well known that it hardly needs reiteration. But what is interesting with this chimera is its involvement of spontaneously-occurring chlorophyll deficiency. Chlorophyll defectives are so very common with the members of the family Gramineae and the genetic background of them is known. However, such instances are few with perennials and their genetic background is rather obscure. From this point of view this chimera is of particular interest although it has no apparent practical value.

Our thanks are due to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, for his interest in the work.

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\* Contribution No. 242 of Indian Institute of Horticultural Research, I.C.A.R., Bangalore-560006.

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## SHORT SCIENTIFIC NOTES

### A.T.D.C. 12 Computer Program which Estimates Mahalanobis $D^2$ Values, Canonical Vectors, and Ranks of $D^2$ Values

A quantitative estimation of genetic diversity is a much needed parameter in selecting suitable parents for controlled crossing work in crop breeding programs. In the lack of a quick quantitative estimation, presently this is done with certain thumb rules for selection. The purpose of this note is to report a computer program which is of importance in analysing the genetic diversity and solving the classificatory problems connected with the selection of genetic diverse germplasm for breeding work. This is a modified and improved version of the program written and documented by Murthy and Arunachalam (1967), and Arunachalam (1967) for an IBM 1620 computer. The present program is written in fortran for a T.D.C. 12 computer currently produced in India. The program uses only 4 K memory, whereas the program given by Murthy and Arunachalam (1967) needs considerable amount of code.

Further information concerning details of the program and instructions for its uses may be obtained from us on request.

We are grateful to Dr. N. K. Anant Rao, Dean, Agriculture; Dr. K. G. Gollakota, Dean, Post-Graduate Studies, Dr. D. D. Pant, Dean, C.B.S.H., for facilities and encouragement.

Second author acknowledges Indian Council of Agricultural Research for the award of a Senior Fellowship.

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November 14, 1973.

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### Late Blight of Potato—A New Report from Maharashtra\*

In the course of his recent mycological excursions at Mahabaleshwar and Panchgani (Alt.: 4,500' and 3,000' resp.), during the month of September, 1973, the writer encountered several fields of

potato imparting a parched appearance and some were completely rotten leaving only barren stumps. On closer examination of the fields at lower altitudes where the disease was less intensive, it was found that the leaves showed water-soaked, ash-coloured circular lesions beginning from the margins ultimately producing a blighting effect. These symptoms were quite distinct from those generally associated with the commonly prevalent Early Blight disease incited by *Alternaria solani*. On critical microscopic examination of the diseased leaves as well as tubers obtained from higher as well as lower altitudes, and isolation in culture, the fungus was determined as *Phytophthora infestans* (Mont.) deBary, the pathogen inciting the notorious LATE BLIGHT. It was found that the disease caused extensive damage varying on complete destruction of the hill grown potatoes at Mahabaleshwar and Panchgani and comparatively less so in the plain-grown potatoes at Wai and other plains (Satara District) situated at the foot of the *Pasarni ghat*.

The Late Blight of potatoes was first reported from India in 1870 from Nilgiris and since then its epidemic outbreaks have been reported to be destructive in almost all potato growing areas including Darjeeling, Hoogly, Simla, Kumaon Hills and Northern plains<sup>1-3</sup> except Bombay (Maharashtra State)<sup>4</sup>.

This is a first report of the introduction of Late Blight of potatoes to Maharashtra State.

Grateful thanks are offered to Prof. M. N. Kamat for his deep interest and helpful suggestions.

Maharashtra Association for A. V. SATHE.

the Cultivation of Science.

Poona-411-004, India.

October 8, 1973.

\* Contribution No. 484 from Department Mycology and Plant Pathology, M.A.C.S., Poona.

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*Psidium guineense* Swartz in Gauhati, Assam

During a botanical collection in greater Gauhati some plants of *Psidium* sp. were collected from the hill slopes in Kharguli area of Gauhati. The plants (A. S. Rao 46048, Hajra 38257 in ASAM) differed from the commonly cultivated *Psidium guajava* L. being stunted undershrubs to shrubs reaching to a height of 1-1.5 m; stem ca 2 cm thick; young leaves ferruginous tomentose on both surfaces, but the old leaves so, only beneath; lateral nerves 5-10 pairs and fruits being 1.5-2 cm in diam. The plants agreed with *Psidium guineense* Sw., a native of Guinea.

Deb (in *Bull. Bot. Surv. India* 3 (1): 87-89, 1961) in recording this plant as naturalised in Tripura has mentioned its original home as New Guinea. There are records of its introduction from Guinea to different countries. A review of the literature shows that recently Backer and Bakhuizen Van den Brink (in *Flora of Java* 1: 334, 1963) have recorded its introduction to Java from tropical America. Similarly Paxton (*A Pocket Botanical Dictionary*, 1849) has also recorded its introduction from Guinea to England as early as 1822. In India Voight (*Hortus Subarhanus Calcuttensis* 46, 1845) has recorded the introduction of this plant into the Company's Botanic Garden (now the Indian Botanic Garden), from the West Indies, where it had been introduced from Guinea.

Considering the common cultural contacts between Bengal and Assam and neighbouring areas including Tripura and till recently the very regular river Traffic on the Brahmaputra river there appears to be a strong probability that the Gauhati plants of *Psidium guineense* Sw. form an introduced and naturalised element and not a native element of the Flora.

It is interesting to note that Lawson (in Oliver's *Flora of Tropical Africa* 2: 436, 1871) has mentioned "*Psidium guineense* Sw., another species cultivated in the West Indies, is said to have been imported there from Guinea but it does not appear that any species have been found in Africa. It is a variety of *P. araca* which is not a native of Africa". This interesting remark leaves the question of the original home of *P. guineense*, itself in doubt.

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Meghalaya, September 25, 1973.

**Botanical Identity of Charcoal from Nagara**

In the excavations at Nagara, Cambay, Gujarat State (Western India) conducted in the year 1965, the archaeologists of the Maharaja Sayajirao University of Baroda unearthed from layer No. 15 (4-15 m

depth) charcoal. The period assignable to this find is 1st Century A.D.B.C. and its botanical identity reveals the existence of *Cedrela toona* Roxb. (Meliaceae) and *Terminalia* sp. similar to *T. tomentosa* W. & A. (Combretaceae).

Santapau (1966) wrote that in many of our floras the tree goes under the name of *Cedrela toona* but Roemer in 1846 showed that the true *Cedrela* trees are exclusively American; our Old World trees have been placed under *Toona*. Thus, he considered the Indian Mahogany, Moulmein Cedar or Toon as *Toona ciliata* Roem., the generally accepted distribution being from Afghanistan, South China, India, Burma and Thailand to New Guinea, Java and Australia. Gillet (1972, in his letter) informs the authors that *Cedrela toona* is found in cultivation in Africa. On the basis of the present archaeological evidence the authors are much inclined to assert that the true, tall trees belonging to the genus *Cedrela* hitherto considered as exclusively American, were existing in India before the 17th century when the specimens with which earlier botanists directly or indirectly dealt were collected!

Locally known as *Sajad*, Cooke (1902) in his *Flora of the Presidency of Bombay* listed the popular 'Ain' tree under the name of *Terminalia tomentosa* Wight & Arn. The identity of the Bombay plant, found in India, Ceylon, Siam and Indo-China, is *T. crenulata* Roth.

The findings reveal the roll of plants in the spread and establishment of human civilization in this part of the country. At Nagara, the last use of the wood of these species was as firewood. This is a fairly common practice to use the planks, trunks and other parts of the timber tree as fuel after its other uses come to an end.

Grateful thanks are due to Dr. D. F. Cutler, Plant Anatomy Section, Jodrell Laboratory, Kew, who was kind enough to help the authors in the determination of the charcoal from Nagara.

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Baroda-2, November 1, 1973.

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## REVIEWS AND NOTICES OF BOOKS

**Non-Equilibrium Thermodynamics in Soil Physics.** By R. C. Srivastava and Raj Pal. (Oxford and IBH Publishing Co. Oxford Bldg., No. 88, Connaught Circus, New Delhi-1), 1973. Pp. xi + 195. Price Rs. 6.75.

Many of the physical phenomena involved in soil-water-plant relationships can be successfully explained with a thermodynamic approach. Much has been written on the application of non-equilibrium thermodynamics to problems in plant systems. A systematic treatment of the subject, demonstrating its usefulness to rate processes in soil systems is long awaited even though many research papers have been published after the first attempt in the right direction by the late Prof. Taylor in early sixties.

The authors with their experience have carefully analyzed the problem and brought out this book, which will be very much useful to students in soil physics. The first four chapters briefly summarized the principles of non-equilibrium thermodynamics, both linear and non-linear. The first and second law of thermodynamics which are essential to understand the thermodynamic theory of irreversible processes are briefly indicated. The reviewer feels that this section could have been better presented. Simultaneous transport phenomena of matter and heat, solute and solvent, and matter and electricity were clearly explained in the last three chapters, in the light of the principles of non-equilibrium thermodynamics.

The book is nominally priced and will definitely be of much use to students and teachers in soil physics.

G. S. R. KRISHNA MURTI.

## ANNOUNCEMENTS

### Indian Academy of Horticultural Sciences, Bangalore-6

With a view to strengthen the requirements of Horticulture and allied sciences in India, an organisation called 'Indian Academy of Horticultural Sciences' has been founded with its headquarters at the Indian Institute of Horticultural Research, Bangalore-6. At the First General Body Meeting of the Academy held at Bangalore, the following office-bearers were elected: President—Dr. K. M. Aiyappa; Vice-Presidents—Dr. K. Ramakrishnan and Dr. M. Nagaraj; Secretary—Dr. G. S. Randhawa; Joint Secretary—Dr. K. S. M. Sastry; Treasurer—Dr. P. S. Rao.

### Award of Research Degrees

The M.S. University of Baroda has awarded the Ph.D. degree in Chemistry to Shri K. B. Shah; Ph.D. degree in Geology to Shri Champaklal Pujalal Shah; Ph.D. degree in Botany to Shri Satyendra Narayanrao.

### Books Received

*The Physics of Phonons.* By J. A. Reissland. (John Wiley & Sons Ltd., London), 1973. Pp. xi + 319. Price £7.00.

*Annual Review of Biochemistry* (Vol. 42). (Annual Reviews, Inc., 4139 El Camino Way, Palo Alto, California 94306), 1973. Pp. vii + 786. Price \$16.00 for U.S.A. and \$16.50 (elsewhere).

*General Entomology* (Second Edition) By. M. S. Mani. (Oxford & IBH Pub. Co., 66, Janpath, New Delhi 1), 1973. Pp. xiii + 597. Price Rs. 18.75.

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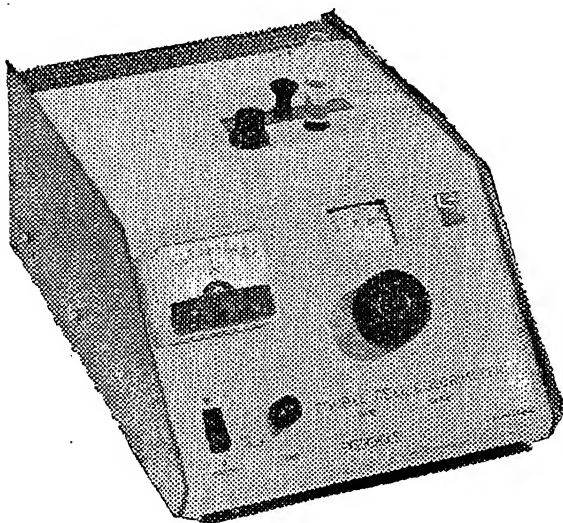
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## ABSTRACT

Within the bonding-antibonding orbital approach of Jha and Bloembergen, it is shown that the bilinear optical susceptibility  $\chi^{(2)}$  for II-VI and III-V semiconductors satisfies a very simple relation in terms of other measurable physical quantities.

## INTRODUCTION

SEVERAL phenomenological and empirical models<sup>1-7</sup> have been proposed to describe the contributions of bound electrons to the bilinear optical susceptibility  $\chi_{ijk}^{(2)}$  defined by

$$P_i = \sum_j \chi_{ij}^{(1)} E_j + \sum_{j,k} \chi_{ijk}^{(2)} E_j E_k + \dots \quad (1)$$

where  $P$  is the optical polarization induced in the medium by an electric field  $E$  and  $\chi_{ij}^{(1)}$  is the linear susceptibility. Although the dispersive part of  $\chi^{(2)}$  may be calculated by using a detailed knowledge of the band structure for the material in a limited range of energy, e.g., using the procedure of Jha and Wynne<sup>7</sup>, the non-dispersive part of  $\chi^{(2)}$  is difficult to obtain theoretically in such an approach. A simple approximation scheme, using tetrahedral bonding orbitals for the ground state of III-V semiconductors, has been considered by Jha and Bloembergen<sup>2</sup> to obtain  $\chi^{(2)}$  in these materials. Flytzanis and Ducuing<sup>3</sup> did a more detailed calculation along these lines, using a sophisticated variational procedure. Phillips and Van Vechten<sup>4</sup> and Kleinman<sup>5</sup> have based their calculations on the dielectric theory developed earlier by Phillips. On similar lines, Tang and Flytzanis<sup>3</sup> have developed the so-called charge-transfer model. Apparently the most successful approach at present seems to be that of Levine<sup>6</sup>, which is based on a semiclassical phenomenological bond-charge model.

In a two-band model involving bonding and antibonding orbital states, we show here that within the Jha-Bloembergen approach,  $\chi^{(2)}$  can be exactly related to  $\chi^{(1)}$  and the effective charge on the adjacent sites in II-VI or III-V compounds, apart from certain overlap terms. Thus, any honest calculation neglecting the overlap must lead to the results described in this paper.

## EXACT RESULTS IN TWO-BAND MODEL

For definiteness, let us consider semiconductors involving two types of atoms A and B, with a

zincblende structure. The unperturbed valence and conduction band wave-functions  $\psi_i^v$  and  $\psi_i^c$  for the bond in the direction  $\hat{l} = a/4 \{1, 1, 1\}$  are

$$\psi_i^v = (1 + \lambda^2 + 2\lambda S_{AB})^{-1/2} (\lambda \phi_i^A + \phi_i^B) \quad (2)$$

$$\psi_i^c = (1 + \lambda^2 - 2\lambda S_{AB})^{-1/2} (\phi_i^A - \lambda \phi_i^B) \quad (3)$$

where  $\phi_i^A$  and  $\phi_i^B$  are normalized  $sp^3$  hybridized orbitals centered on atoms A and B,  $S_{AB}$  is the overlap between these two orbitals and  $\lambda$  is a parameter which is directly related to the effective charge at each site. Within a two-band model, one has<sup>2</sup>

$$x_{xx}^{(1)} = (2e^2 n/Eg) |\langle x \rangle_{vc}|^2 \quad (4)$$

$$x_{xyz}^{(2)} = -(3e^3 n/Eg^2) |\langle x \rangle_{rc}|^2 \langle \langle x \rangle_{rc} \dots \langle x \rangle_{vc} \rangle \quad (5)$$

where  $n$  is the valence electron density and  $Eg$  is the energy gap between the valence and conduction band states. With our wave-functions, the dipole matrix elements are

$$\begin{aligned} \langle x \rangle_{rc} &= \{(1 + \lambda^2)^2 - 4\lambda^2 S_{AB}^2\}^{-1/2} \\ &\times \{\lambda (x_{BB} - x_{AA}) + (\lambda^2 - 1) x_{AB}\} \end{aligned} \quad (6)$$

$$\begin{aligned} \langle x \rangle_{vv} &= \{(1 + \lambda^2 + 2\lambda S_{AB})^{-1} \\ &\times (\lambda^2 x_{AA} + x_{BB} + 2\lambda x_{AB}) \end{aligned} \quad (7)$$

$$\begin{aligned} \langle x \rangle_{cc} &= (1 + \lambda^2 - 2\lambda S_{AB})^{-1} \\ &\times (\lambda^2 x_{BB} + x_{AA} - 2\lambda x_{AB}) \end{aligned} \quad (8)$$

where  $x_{ij}$  is the matrix element of  $x$  between the  $sp^3$  orbitals centered at sites I and J. Eqs. (4-8) immediately lead to our main result

$$\begin{aligned} x_{xyz}^{(2)} &= \frac{3e}{2Eg} \chi^{(1)} \left[ \frac{(1 + \lambda^2)}{(1 + \lambda^2)^2 - 4\lambda^2 S_{AB}^2} \right. \\ &\times \{(1 + \lambda^2)^2 - 4\lambda^2 S_{AB}^2\}^{1/2} \\ &\times \frac{(1 - \lambda^2)}{\lambda} \langle x \rangle_{rc} + \frac{(1 + \lambda^2)^2}{\lambda} \\ &\times x_{AB} - 2\lambda S_{AB} (x_{AA} + x_{BB}) \left. \right\} \end{aligned} \quad (9)$$

Note that if one neglects overlaps  $S_{AB}$  and  $x_{AB}$  and uses the  $f$ -sum rule

$$(2mEg/\hbar^2) |\langle x \rangle_{vc}|^2 = 1 \quad (10)$$

\* On leave from the Institute of Science, Nagpur.

TABLE I

Crystal	$\chi^{(1)}$	$n (10^{23} \text{ cm}^{-3})$	$\lambda$	$\chi_{xyz}^{(2)}$ ( $10^{-6}$ esu) (Theor.)	$\chi_{xyz}^{(2)}$ ( $10^{-6}$ esu) (expt.)	
ZnS	..	0.33	2.02	0.41	0.25	$0.15 \pm 0.04$ [9]
ZnSe	..	0.39	1.76	0.44	0.34	$0.37 \pm 0.14$ [9]
ZnTe	..	0.50	1.42	0.46	0.57	$0.44 \pm 0.16$ [9]
CdTe	..	0.49	1.18	0.46	0.63	$0.80 \pm 0.30$ [9]
GaAs	..	0.79	1.78	0.66	0.53	$1.80 \pm 0.6$ [9]; $0.90 \pm 0.3$ [10]
InAs	..	0.90	1.44	0.66	0.73	$2.00 \pm 0.6$ [9]
InSb	..	1.17	1.18	0.69	1.27	$3.30 \pm 0.7$ [9]
GaP	..	0.65	1.97	0.65	0.31	$0.50 \pm 0.1$ [9]

one obtains a very important expression for  $\chi^{(2)}$

$$\chi_{xyz}^{(2)} = (3/\sqrt{8}) (m/e^2 \hbar^3 n^3)^{1/4} \times (\chi^{(1)})^{7/4} (1 - \lambda^2)/\lambda \quad (11)$$

relating  $\chi_{xyz}^{(2)}$  to known physical quantities. Similar relations may be obtained for other materials with different structures.

Using known values of  $\chi^{(1)}$  and the values of  $\lambda$  determined from experimental results on phonon frequencies and the Szigeti formula with the local field correction factor (these are also in close agreement with Coulson, Redei and Stocker<sup>8</sup>), we give values of  $\chi^{(2)}$  for II-VI and III-V compounds, in Table I. A comparison of our theoretical values with known experimental results shows that there is an excellent agreement for II-VI compounds. However, except for GaP, the agreement is poor for III-V compounds. We would like to stress again that our conclusion is independent of the explicit forms of the orbital wave-functions, and any calculation which neglects overlaps and considers only two bands must lead to these results. Since the role played by overlap integrals, if any, must be similar in both II-VI and III-V compounds, for explaining the discrepancy in the microscopic calculations for III-V compounds, it is perhaps necessary either to modify the effective values of  $\lambda$  in order to avoid overestimating the local field correction factors, or to consider more than two bands.

## ACKNOWLEDGEMENT

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## THE CIRCULAR DICHROISM OF SPHINGOMYELIN

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### ABSTRACT

Circular dichroism (CD) spectra of sphingomyelin (SM) solution in methanol at room temperature and SM lyotropic dispersions in the temperature range of 4° to 60° C were obtained in the region of 350 nm to 200 nm. Variation of rotational strength of main CD bands at 210 nm and 290 nm suggests considerable conformational mobility within SM dispersions and also the possible existence of phase transitions. Possible biophysical implications of these results are discussed.

### INTRODUCTION

IN the past several years much attention has centered upon the studies of functional and structural changes in biomembranes<sup>1-5</sup>. These changes are usually believed to be related to the capability of membrane lipids and lipid-water systems to exist in a variety of different structures, or phases, depending upon, among other things, water content and temperature<sup>6-8</sup>. Most of the lipid-water systems have been studied as a function of water content and temperature by calorimetric, X-ray diffraction, IR, NMR, ESR studies, etc. It has been shown that membrane lipids may undergo one or more thermal phase transitions. Sphingomyelin is an important component of neuronal membrane systems, is known to be relatively stable as compared with glycerophospholipids, and is thought to be a structural lipid in membranes<sup>9</sup>. There are but a few studies on phase transitions in sphingomyelin. Chistyakov and Usov'tseva<sup>10</sup> have studied the thermotropic liquid crystalline behavior of sphingomyelin and have found an endotherm at about 115° C and a smectic liquid crystalline phase upto 196° C. X-ray studies<sup>11</sup> on the phase transitions of sphingomyelin-water phases at 40° C as a function of lipid concentration, showed that at about 25° C sphingomyelin starts crystallizing and at 45° C a lamellar phase is obtained. At low concentrations (less than 40%) a state of cogel or colloidal suspension is obtained. Other studies have dealt with phase transitions in sphingomyelin-cholesterol films<sup>12</sup> and aqueous dispersions of sphingomyelin<sup>13</sup> using ESR and calorimetric techniques.

Valuable information concerning molecular structure and conformation can often be obtained from

circular dichroism (CD) studies<sup>14-16</sup>. CD spectra are usually very sensitive to conformational changes, and membrane phase transitions may involve such changes. The exact nature of the structural reorganization and the forces involved during such thermal transitions are not well known. There is some evidence<sup>17-18</sup> that the polar head groups in membrane lipids have a higher degree of rotational freedom above the transition temperature. The possibility of conformational changes in the polar head group arrangement during phase transitions has been shown by the recent work of Trauble<sup>8,19</sup>. X-ray, IR, NMR and DTA studies<sup>6,7</sup> lead to the conclusion that the hydrocarbon chains are in an ordered quasi-crystalline arrangement below the transition temperature, while above the transition point they are in a "more fluid state" with considerable internal motion. Some investigators<sup>20,21</sup> postulate that the hydrocarbon chains are relatively ordered and possess a limited degree of rotational freedom corresponding to the structural organization found in condensed monolayers. Other workers<sup>6</sup> suggest that the chains are liquid-like and highly disordered which results in considerable flexing, coiling and twisting of the chains. If the phase transitions involve order-disorder structural modifications in the hydrocarbon chains and/or polar head-groups, these structural changes may possibly be reflected in the dependence of CD spectra upon temperature. No detailed CD or ORD study seems to have been carried out on aqueous dispersions of sphingomyelin as a function of temperature. In his classical experiments, Levene<sup>22</sup> reported specific rotations  $[\alpha]_D$  for different preparations of brain sphingomyelins dissolved in equal parts of chloroform and methylalcohol, varying from 7.53° to 8.73°, while Walz<sup>23</sup> gave a value for  $[\alpha]_D$  of +5.5°. Sano<sup>24</sup> found that in pyridine the optical rotation of sphingomyelin is strongly dextro-rotary above 40° C, the value being  $[\alpha]_D = +13.82^\circ$ . However, when

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the solution is cooled below 40° C the optical activity changes from a dextro- to levo-rotation. Considering these scattered results it appeared desirable to collect a more complete set of CD data for sphingomyelin aqueous dispersions as a function of temperature under conditions more relevant to biomembranes. Therefore, this report is primarily concerned with a CD study of the organization of bovine brain sphingomyelin aqueous dispersions and their dependence upon temperature in the range of 4° to 60° C in the ultra-violet region of 350 nm to 200 nm.

## MATERIALS AND METHODS

The bovine brain sphingomyelin used in this study was obtained from Sigma Chemical Company. A JASCO-J 20 automatic recording spectropolarimeter with a CD attachment was used for recording the CD spectra. The pen gain was calibrated using  $d_{10}$ -camphor sulphonic acid<sup>25</sup>. Measurements were made at six different concentrations of sphingomyelin dispersions in distilled water (maximum 10 mg/ml), to verify conformity with Beer's law. CD spectra for sphingomyelin solution in methanol (20 millimol.) was also recorded at room temperature. All the measurements were made in a thermostat cell holder supplied by the manufacturer. The temperature was regulated with a Nestlab T3 circulating thermostat bath. The CD spectra for all samples were measured in a 5 mm pathlength cell at a sphingomyelin estimated concentration of about 1 mg/ml at 4°, 15°, 20°, 27°, 37°, and 60° C. The accuracy of the temperature measurement was  $\pm 0.5^\circ$  C. A series of CD measurements on a single sample at all temperatures was completed in a single day. The reproducibility of repeated measurements at a given temperature on a given sample demonstrated the absence of artifacts stemming from water evaporation or the aggregation and/or precipitation of sphingomyelin micells. Reproducibility of the data was checked with three different sample preparations of sphingomyelin dispersions with a view to eliminate from consideration contributions to the CD spectra arising from light scattering and flattening-broadening due to the particulate nature of the dispersion<sup>14</sup>.

where  $[\theta]$  is the molar ellipticity in the units of  $\text{deg cm}^2 \text{decimole}^{-1}$ ,  $S$  is the scale factor in  $\text{deg cm}^{-1}$ ,  $M$  is the gram-molecular weight,  $C$  is the concentration of sphingomyelin in  $\text{mg ml}^{-1}$ ,  $L$  is the pathlength in mm, and  $\Delta D$  is the recorded pen deflection on the chart in cm. The molecular weight of sphingomyelin was taken as 800 daltons. The CD data were not corrected for the refractive index dispersion of the solvent. Corrections for the thermal expansion of the solvent and for background were found to have negligible contributions with respect to the total CD observed, and they were not considered for the purpose of final calculations. The CD curves were averaged and approximated by means of smoothed-out curves. The main CD bands were approximated by symmetrical Gaussian curves<sup>21</sup> and the rotational strength of a given optically active transition was calculated by the expression<sup>14</sup>.

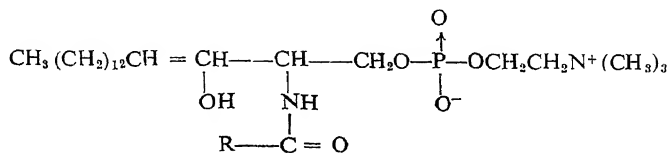
$$R_k = 1.23 \times 10^{-12} [\theta]_k^\circ \Delta_k^\circ / \lambda_k^\circ \quad (\text{CGS units})$$

where  $R_k$  is the rotational strength,  $\lambda_k^\circ$  is the maximum wavelength,  $\Delta_k^\circ$  is the half band-width at  $[\theta]_k^\circ$ , and  $[\theta]_k^\circ$  is the molar ellipticity at  $\lambda_k^\circ$ .

## RESULTS AND DISCUSSION

To begin, the CD spectra of sphingomyelin-aqueous solution dispersions at high concentrations do not conform with Beer's law. At concentrations greater than 5 mg ml<sup>-1</sup>, for example, the CD band in the region of 210 nm, and the spectra below it are hindered by the high optical absorbance and the unfavourable CD/A ratio. Also, gradually during the course of an experiment, aggregation and precipitation of sphingomyelin-micelles begins. In view of these findings a low concentration of sphingomyelin dispersion, about 1 mg ml<sup>-1</sup>, was chosen for detailed study. Under these conditions the sphingomyelin molecules probably are in a highly hydrated state.

The general features of CD spectra of sphingomyelin aqueous dispersions at room temperature are: A weak positive Cotton effect around 290 nm and a more pronounced negative Cotton effect around 210 nm, with a cross-over between 240-260 nm. The structural formula for sphingomyelin is shown below:



The ellipticity data were calculated from the equation :

$$[\theta] = S \times M \times \Delta D/C \times L$$

where R is the residue of a fatty acid of length usually 18:0, 24:0 or 24:1. The two chromophores, the amide and phosphoryl groups are

possibly, some helical nature of the side chains may contribute to the CD spectra of sphingomyelin.

The amide chromophore in an asymmetric environment is known to give an optically active transition near 210 nm<sup>16</sup>. Lewis *et al.*<sup>27</sup> have reported an optically active transition at 210 nm due to an arylphosphoryl chromophore in methyl phosphinates. Urry *et al.*<sup>28</sup> found that phosphatidylcholine and phosphatidyl-ethanolamine, both of which contain a phosphoryl group similar to that found in sphingomyelin, give a positive CD peak at 218 nm and a negative peak at  $192 \pm 2$  nm in trifluoroethanol. These authors have attributed the 218 nm CD extremum of these phospholipids to the  $n \rightarrow \pi^*$  transition in the ester groups. However, Morre and Wetlaufer<sup>29</sup> found no evidence for dichroic bands for beef brain phosphatidylserine dissolved in diethylether and also for a sonicated dispersion of egg yolk lecithin over the wavelength range 250 nm—210 nm. In view of these findings, the 210 nm negative CD band in sphingomyelin may be attributed to the amide chromophore. A red shift from 210 nm to 240 nm and a considerable (one order of magnitude) decrease in the ellipticity was observed for sphingomyelin solution in methanol. The ellipticity of 290 nm band was also decreased by about 50% of the value observed in lyotropic dispersion, but its position remains unchanged. Dramatic effects, due to the nature of solvents, have been observed with numerous other chromophores<sup>16</sup>. There is no conclusive evidence concerning the chromophore responsible for 290 nm CD band of sphingomyelin, and the relative contributions of the various chemical groups to this transition are unknown; one may possibly attribute this transition, in part, to the amide chromophore and the polar group. Some contribution to the optical activity around 210 nm from side chains may or may not be present.

The temperature dependence of the CD spectra of sphingomyelin aqueous dispersions is shown in Fig. 1, and the variations in the CD parameters have been tabulated in Table I. Variation of rotational strength for both transition bands as a function of temperature is shown in Fig. 2. The rotational strengths of both the 210 nm and 290 nm transitions do not change monotonically with increasing temperature. An attempt to describe the observed temperature dependence of the rotational strength in terms of one or two conformers<sup>30</sup> was not successful. A sudden decrease in the rotational strength for both transition bands is observed in the temperature range of 15° to 20° C. Between 20° and 27° C again there is a decrease in rota-

tional strength of both bands, but more so for the 290 nm transition. Between 27° and 37° C the rotational strength for the 290 nm transition does not change appreciably, whereas the rotational strength for the 210 nm transition band changes dramatically. Between temperatures 37° and 60° C the rotational strength for the 290 nm transition goes on decreasing monotonically while the rotational strength of the 210 nm transition does not vary appreciably between temperatures 37° and 50° C, but does decrease between 50° and 60° C. Some of the temperature ranges in which there is considerable and sudden change in the rotational strength of either of the two transition bands are identical to temperature ranges where other workers report that phase transitions occur. The remaining temperature ranges corresponding to changes in rotational strengths may also correspond to

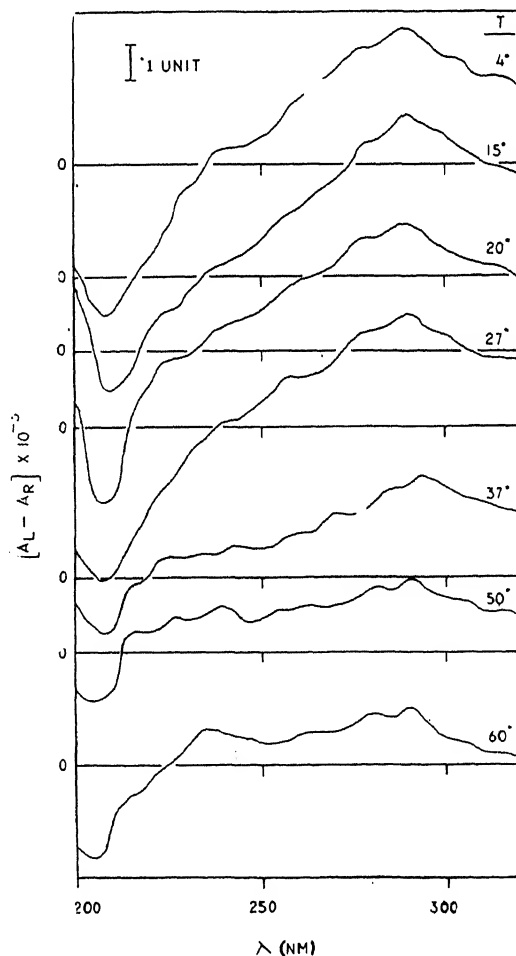


FIG. 1. CD spectra of sphingomyelin dispersions in aqueous solution as a function of temperature.

TABLE I

Sl. No.	Temp. °C	Positive band				Cross over nm	Negative band			
		$\lambda_k^\circ$ nm	$\Delta_k^\circ$ nm	$[\theta]_k^\circ$	$R_k \times 10^{42}$		$\lambda_k^\circ$ nm	$\Delta_k^\circ$ nm	$[\theta]_k^\circ$	$R_k \times 10^{42}$
1	4	288	18	0.560	4.263	235	208	14	-0.656	-6.114
2	15	289	20	0.672	5.604	235	210	13	-0.480	-3.654
3	20	288	22	0.544	5.115	232	207	13	-0.640	-4.872
4	27	290	17	0.464	3.288	240	208	14	-0.656	-5.358
5	37	293	18	0.432	3.168	220	208	6	-0.240	-0.852
6	50	290	16	0.320	2.193	212	205	9	-0.208	-1.095
7	60	289	13	0.240	1.341	225	205	9	-0.384	-2.070

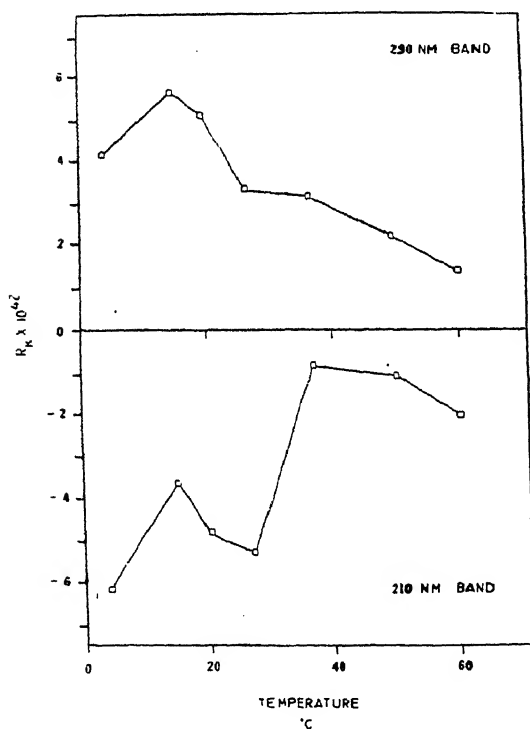


FIG. 2. Plots of  $R_k \times 10^{42}$  versus temperature for the maximum strengths of the 290 nm and 210 nm transition bands of the CD spectra of sphingomyelin dispersions in aqueous solution.

rearrangements of structure, or phase transitions, and do not seem to have been appreciated previously. If the two optically active transitions in sphingomyelin can be attributed to the amide and phosphoryl chromophores, and if the magnitude of the spectral changes are proportional to the extent of structural

modification, then the relative importance and contributions of these two groups in various phase transitions may be gleaned from Fig. 2. Also, the relative conformational mobility of the hydrocarbon side chains and the polar head groups as a function of temperature may be assessed from the same figure. Figure 1 shows that the 290 nm band broadens with increasing temperature, especially above 27°C, at the expense of the negative 210 nm band. This may be due to some coupling between the rotational freedom of the two chromophores due to interactions occurring between various segments of the sphingomyelin molecule.

The results of the present study demonstrate, under the experimental conditions mentioned earlier, that the sphingomyelin molecule may have considerable conformational mobility, and sudden changes in the rotational strength of its optically active transitions seems to correspond to structural rearrangements or phase transitions. Under the present experimental conditions, sphingomyelin is most likely to adopt the lamellar structure. The possibility of the phospholipid aqueous dispersions, existing in several forms, (i) spherical micelle, (ii) cylindrical micelle, (iii) bilayer, (iv) lamellar and (v) bubble membrane, has been pointed out<sup>31</sup>. These different micellar structures are likely to possess different CD spectra. Several theoretical<sup>31,32</sup> and experimental<sup>33</sup> studies on the transformations among some of these structural forms in the liquid crystal phases have been made. Since the relative contribution of the various chemical groups in sphingomyelin to the CD spectrum is, at present, not certain, it is not possible to deduce from the available data the exact structure of the various lamellar arrangements which give rise to a particular CD curve at a given temperature.

TABLE I

Effect of O-R potential on the growth of *E. histolytica* strain 200:NIH in TP-S-1-medium containing cysteine without ascorbic acid. An inoculum of 5,000 amoebae/ml of the medium was used

Seitz filtered cysteine in TP-S-1- medium %	O-R poten- tial mv	No. of amoebae/ml of the medium after days*								
		1	2	3	4	5	6	7	8	9
0.3	- 300	10,000	29,000	70,000	110,000	200,000	260,000	320,000	..	..
0.2	- 290	10,000	20,000	65,000	100,000	..	210,000	300,000	..	..
0.1	- 250	9,000	20,000	50,000	70,000	100,000	150,000	200,000	..	..
0.05	- 200	8,000	17,000	34,000	50,000	70,000	100,000	150,000	..	..
0.025	- 135	5,500	9,900	16,000	20,000	30,000	50,000	60,000	..	..
Autoclaved cysteine in TP-S-1- medium %										
0.3	- 183	8,000	18,000	50,000	..	..	180,000	260,000	250,000	20,000
0.2	- 170	8,000	16,000	40,000	..	..	160,000	260,000	240,000	16,000
0.1	- 153	7,000	14,000	28,000	..	..	100,000	150,000	150,000	10,000
0.05	- 136	6,400	12,000	16,000	..	..	60,000	90,000	70,000	5,000
0.025	- 120	4,200	9,000	14,000	..	..	26,000	10,000	5,000	Nil

\* Mean count from duplicate tubes.

Diamond<sup>11</sup> in 1961 used autoclaved 0.1% cysteine and 0.02% ascorbic acid in a diphasic axenic culture medium to grow *E. histolytica* (strain 200:NIH). It has been shown by Singh, Das and Dutta<sup>9</sup> that it is dangerous to use cysteine + ascorbic acid in axenic TP-S-1-medium because this combination leads to a shift of O-R potential towards positive side which is lethal to amoebae when the medium is stored for 10 days or more. Moreover, the negative O-R potential produced by the above autoclaved combination of cysteine + ascorbic acid is not sufficiently low for the rapid growth of amoebae. It has been clearly shown in the present investigation that strongly negative O-R potential is necessary to cut down the lag phase of amoebae inoculated into axenic medium and to obtain the maximum population as has been observed in the case of *E. histolytica* growing with bacterial associates<sup>2-4</sup>.

The authors are grateful to Dr. L. S. Diamond for supplying axenic culture of *E. histolytica* strain 200:NIH.

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## LETTERS TO THE EDITOR

CHEMICAL INVESTIGATION OF LICHEN :  
CYCLOPLACA ALMORENSIS : ISOLATION OF  
PHYSCION AND LECANORIC ACID

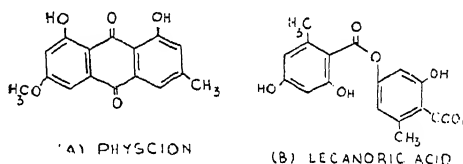
*Cycloplaca almorensis* (Ras)-Awasthi belongs to the sub-class—Ascolichen, series—Gymnocarpinae and sub-series—Cyclocarpinae and family—Cycloplacaceae. It occurs as reddish-brown circular spots, 4-8 mm in diameter, on epidote granite rock in the Adikmet area of Hyderabad. The pieces of rocks were collected and carefully broken down into small pieces. The pieces containing lichen were extracted with large quantities of ether in the cold. The ether suspension was left overnight. The deep yellow coloured ether extract was then filtered and concentrated to a small volume. A brown product that separated was filtered and recrystallised from ethyl alcohol. The first batch obtained after recrystallisation gave orange-red crystals of compound 'A' and the second yielded pale yellow crystals of compound 'B'.

Compound A on repeated recrystallisation with benzene afforded orange-red needles, m.p. 206° C, which by TLC using a solvent mixture benzene-dioxane-glacial acetic acid (90 : 25 : 4) indicated it to be a single substance. It gave a purple coloured solution with sodium hydroxide and is insoluble in sodium carbonate. The orange-red crystals on dissolving in concentrated sulphuric acid gave a blood red colour. It has an absorption at 1660  $\text{cm}^{-1}$  in IR, indicating the presence of chromone carbonyl, chelated hydroxyl at 3300  $\text{cm}^{-1}$  and 1310  $\text{cm}^{-1}$  for C—O—C stretching. In UV, two maxima were observed at 271 nm ( $\log \epsilon$  4.1) and 285 nm ( $\log \epsilon$  4.1).

Compound B was repeatedly triturated with benzene until the solvent was colourless and then filtered. The colourless substance thus obtained was then recrystallised from alcohol in the form of colourless needles, m.p. 175° C, which by TLC with benzene : ethylacetate (1 : 1) mixture gave a single spot. The substance gave reddish violet colouration with ferric chloride. It is soluble in sodium bicarbonate solution and could be reprecipitated on acidification. The compound gave red blood colouration with bleaching powder and on the addition of excess of the reagent, decolourisation took place. The compound exhibited a broad and intense absorption at 1700  $\text{cm}^{-1}$  of carbonyl, indicating it to be an acid. Its UV spectra indicated two absorption maxima at 272 nm ( $\log \epsilon$  4.0) and 305 nm ( $\log \epsilon$  3.9).

Compound A was compared with the authentic sample of physcion and found to be identical, by superimposable IR spectra and identical UV spectrum. Compound B was found to be identical with an authentic sample of lecanoric acid, as its IR was superimposable and UV spectrum was identical.

Thus the two compounds isolated from *Cycloplaca almorensis* have been characterised as physcion and lecanoric acid. This appears to be the first instance of the chemical investigation of Cyclocarpinae series where the co-occurrence of physcion and lecanoric acid is observed.



We convey our thanks to Prof. M. R. Saxena, Head, Botany Department, Osmania University, for kindly supplying the lichen material and characterisation.

Our thanks are also due to Dr. P. S. Rao, Warangal, for kindly sending us the authentic samples of physcion and lecanoric acid.

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MINOR ISOFLAVONOID GLYCOSIDES OF THE  
STEM BARK OF *DALBERGIA PANICULATA*—  
ISOLATION OF A NEW C-GLYCOSIDE

In an earlier communication<sup>1</sup> the isolation of biochanin-A, formononetin and paniculatin from the bark of *Dalbergia paniculata* was reported. The present one deals with other minor glycosidic constituents left in the mother liquor of the methanol extract after the separation of paniculatin. The mother liquor was concentrated and subjected to column chromatography over silica gel, employing solvents of increasing polarity. The fractions were further purified by preparative TLC using silica gel. Three pure compounds were obtained, and marked (A), (B) and (C). All of them gave colour tests for isoflavones and were found to be glycosidic in nature.

Compound (A) obtained from benzene-ethylacetate (3 : 1) eluate followed by preparative TLC

using silica gel, (EtOAc-H<sub>2</sub>O-MeOH 40 : 5.4 : 6.6) crystallised as colourless thin rectangular prisms from methanol, m.p. 205–6°;  $\lambda_{\text{max}}^{\text{EtOH}}$  262 nm. On acid hydrolysis it furnished biochanin A and glucose as the aglycone and sugar respectively. It was thus identified as biochanin-7-O-glucoside (sissotrin) and the identity was confirmed by comparison with an authentic sample<sup>2</sup> (m.m.p., co-TLC and superimposable I.R.).

Compound (B) was obtained later from benzene-ethylacetate (3 : 1) eluate and was separated from compound (A) by preparative TLC. It crystallised from methanol as colourless tiny rectangular prisms, m.p. 242–3° and gave no ferric colour;  $\lambda_{\text{max}}^{\text{MeOH}}$  261 nm, with no shifts on the addition of NaOAc or AlCl<sub>3</sub>. On acid hydrolysis it gave formononetin as the aglycone and only glucose could be detected as the sugar residue. Since its R<sub>f</sub> value was very close to that of sissotrin, it was inferred to be the 7-O-glucoside of formononetin. All its properties were in full agreement with those reported for ononin<sup>3</sup>.

Compound (C) was a new glycoside obtained from pure ethylacetate eluate and it crystallised from methanol as colourless glistening plates, m.p. 286–7°. It gave a green ferric colour;  $\lambda_{\text{max}}^{\text{MeOH}}$  261 nm, with no shift with NaOAc but a shift to 270 nm on addition of AlCl<sub>3</sub>. It did not undergo hydrolysis with mineral acid even under prolonged heating (5 hrs) suggesting that it was a C-glycosyl compound.

It formed a hexaacetate, m.p. 128–30° whose N.M.R. spectrum in CDCl<sub>3</sub> showed the following: four alcoholic acetoxylys with  $\delta$  1.72 (3H), 2.00 (3H), 2.05 (6H), two phenolic acetoxylys  $\delta$  2.30 (3H) and 2.40 (3H) and one methoxyl group  $\delta$  3.95 (3H), one A ring proton  $\delta$  6.65, a *p*-disubstituted benzene ring (ring B)  $\delta$  7.15, 7.50 (*d*, J = 9Hz) and the isoflavone C<sub>2</sub>-H at  $\delta$  7.90. The above spectral data and the properties of compound C suggested that it could be a C-glucoside of genistein monomethyl ether. The methoxyl group could be located at the 7-position in view of the absence of shift in U.V. with sodium acetate. This was also supported by the agreement of N.M.R. signals due to the protons at 3', 5'-position in the acetate ( $\delta$  7.15) with those in sissotrin acetate ( $\delta$  6.91) and paniculatin acetate ( $\delta$  7.10) showing that the 4'-position has acetoxy. The location of the C-glucosyl residue at 8-position could also be inferred from the N.M.R. as the 2''-acetoxy signal<sup>4–6</sup> was found at  $\delta$  1.72 as observed in 8-C-glucosyl flavones, the upfield shift being attributed to the A-ring. In known 6-C-glucosyl

compounds, the signal due to this acetoxy occurs definitely at lower fields ( $\delta$  1.85). Thus compound (C) could be assigned the structure as 8-C-glucosyl prunetin.

The mass spectrum of compound C confirmed the above conclusion. A feature of the mass spectrum is the presence of peaks at M–120, M–133 and M–149, which have been found to be characteristic of 8-C-glucosides and differentiated them from 6-C-glucoside. Absence of peak at *m/e* 132 corresponding to *p*-methoxy phenyl acetylene ion also confirmed the location of methoxyl at the 7-position.

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#### TRITERPENOIDS FROM THE STEM-BARK OF *DALBERGIA SERICEA*

*Dalbergia sericea* (Family : Leguminosae) is a tree found in Eastern Himalayas and Assam. No chemical work seems to have been done in the past. The petroleum ether extract of the stem bark (1 kg) on column chromatography over silica gel yielded four crystalline compounds A, B, C and D which constitute some rare triterpenes.

Compound A (450 mg) crystallised from chloroform-methanol as colourless needles, m.p. 168°,  $[\alpha]_D^{25}$  –13.6° (c, 0.690 in CHCl<sub>3</sub>). It analysed for C<sub>32</sub>H<sub>52</sub>O<sub>2</sub> (M: 468);  $\nu_{\text{max}}^{\text{KBr}}$  1745, 1640, 1140, 825 and 805 cm<sup>–1</sup>; NMR ( $\delta$  values), 0.75 (s, 3H), 0.87 (s, 12H), 0.97 (s, 3H), 1.60 (s, 3H), 1.67 (s, 3H), total of 8C-methyls; 2.01 (s, 3H, CH<sub>3</sub> COO), 4.50 (m, 1H, H–C–OAc) and 5.01 (m, 1H, HC=C). Mass spectrum had peaks at *m/e* 468 and 68 (base peak). Spectral data indicated the presence of an acetate group and it was confirmed by saponification with 10% methanolic KOH yielding an alcohol, which crystallised from chloroform-methanol as colourless needles, m.p. 133–34°.

It analysed for  $C_{30}H_{50}O$  ( $M^+$  426),  $[\alpha]_D + 6.2^\circ$ . ( $c$ , 0.840 in  $CHCl_3$ );  $\nu_{max}^{BrK}$  3400  $cm^{-1}$  (OH). The alcohol on reacylation with acetic anhydride-pyridine for 24 hours at room temperature gave back compound A.

The above data led to the identification of compound A as tirucallol acetate; this is the first report of its occurrence in nature and therefore its spectral data are given above. The alcohol tirucallol was earlier reported from *Euphorbia tirucalli*<sup>1</sup>. Direct comparison of its acetate kindly provided by Prof. D. H. R. Barton with the compound A using co-TLC, m.m.p., and superimposable IR confirmed its identity.

**Compound B** (120 mg) crystallised from chloroform-methanol as colourless needles, m.p. 222–23° and  $[\alpha]_D + 58.3^\circ$  ( $c$ , 0.380 in  $CHCl_3$ ). It analysed for  $C_{30}H_{50}O$  ( $M^+$  426),  $\nu_{max}^{KBr}$  3540, 1635, 860 and 800  $cm^{-1}$ . NMR ( $\delta$  values): 0.83–1.20 (8 C-methyls), 3.42 (m, 1H, H–C–OH) and 5.50 (m, HC=C). Mass spectrum had peaks at  $m/e$  426 ( $M^+$ ) and 95 (base peak). It formed an acetate on treatment with acetic anhydride-pyridine, m.p. 187–88° and  $[\alpha]_D + 74.1^\circ$  ( $c$ , 0.624 in  $CHCl_3$ ). On oxidation with Jones' reagent compound B gave a ketone, m.p. 239–40°.

The above data led to the identification of compound B as the comparatively rare triterpene, glutinol and this was confirmed by the identity of the ketone with an authentic sample<sup>2</sup>.

**Compound C** (380 gm) has been identified as  $\beta$ -sitosterol by direct comparison with an authentic sample.

**Compound D** (250 mg) crystallised from chloroform-methanol as colourless needles, m.p. 283–85°.  $\nu_{max}^{KBr}$  3500, 1480, 1360, 1035, 995 and 812  $cm^{-1}$ . NMR ( $\delta$  values): 0.83–1.20 (8 C-methyls) and 5.50 (m, HC=C). It formed an acetate on treatment with acetic anhydride-pyridine for 48 hours, m.p. 304–5°;  $[\alpha]_D + 11^\circ$  ( $C$ , 0.73 in  $CHCl_3$ ). On oxidation with Jones' reagent the compound gave a ketone, m.p. 235–37°. These data led to its identification as taraxerol which was confirmed by direct comparison with an authentic sample<sup>3</sup>.

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## ALKALOIDS FROM THE FRUITS OF *PIPER AURANTIACUM* WALL (PIPERACEAE)

*Piper aurantiacum* Wall is one of the important species belonging to the botanical family Piperaceae and is valued as a medicinal plant<sup>1</sup>. It was reported by Arora *et al.*<sup>2</sup> that no alkaloids were present in the dried fruits of *P. aurantiacum*. But during pharmacological study of *P. aurantiacum*, Banerjee *et al.*<sup>3</sup> reported the presence of alkaloids by paper chromatographic examination. However, no detailed chemical examination was done so far. In the present investigation we have been able to isolate three alkaloids piperine, piperettine and sylvatine besides  $\beta$ -sitosterol from the hexane extract of the dried fruits of *P. aurantiacum* by column chromatography over silica gel.

The dried fruits of *P. aurantiacum* were powdered and extracted thoroughly with hexane. The hexane extract on evaporation under vacuum gave yellow waxy material. It was extracted with methanol and the methanol soluble fraction was concentrated and hexane added. When left in the refrigerator for three days, a yellow crystalline solid separated. The yellow solid showed two spots when subjected to thin layer chromatography on silica gel plate, the upper spot being the major one. The solid mixture was adsorbed on a column of silica gel set up with benzene and eluted successively with benzene, benzene : ethyl acetate (4 : 1) and (1 : 1). The benzene : ethyl acetate (4 : 1) fraction, on concentration, gave pale yellow crystalline solid. It crystallised from benzene as pale yellow rhombic crystals, m.p. 132° (Found : C 71.90; H 6.59 and N 4.72;  $C_{17}H_{19}O_3N$  requires C 71.93; H 6.67 and N 4.91%). UV  $\lambda_{max}^{EtOH}$  343 nm; IR  $\nu_{max}^{KBr}$  3000, 2970, 1640, 1620, 935  $cm^{-1}$ . It was identified as piperine by m.m.p., superimposable IR, NMR and co-tlc with authentic sample.

From benzene : ethyl acetate (1 : 1) fraction the solvent mixture was distilled off and hexane added, when a yellow solid separated. It crystallised from benzene : hexane as yellow needles, m.p. 148° (Found : C 73.60, H 7.10 and N 4.70;  $C_{19}H_{21}O_3N$  requires C 73.30, H 6.80 and N 4.50%). It was identified as piperettine by m.m.p., UV  $\lambda_{max}^{EtOH}$  (364 m $\mu$ ) and co-tlc with authentic sample.

The filtrate from the methanol soluble fraction was adsorbed on a column of silica gel set up with benzene and eluted successively with benzene, benzene : ethyl acetate (9 : 1), (4 : 1) and (1 : 1). The benzene eluant gave oily material only. The earlier fractions from benzene : ethyl acetate (9 : 1) eluant were concentrated and methanol added. A white crystalline solid separated which was crystallised from methanol as colourless needles,

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m.p. 138°. It gave positive Liebermann-Burchard test. Its acetate melted at 134°. It was identified as  $\beta$ -sitosterol by m.m.p. and co-tlc with authentic sample.

The latter fractions of benzene:ethyl acetate (9:1) were evaporated and hexane added. A white crystalline solid separated which was crystallised from benzene:hexane as colourless needles, m.p. 116–17° (Found: C 75.13, H 8.25 and N 3.58;  $C_{24}H_{33}O_3N$  requires C 75.19, H 8.62 and N 3.66%).  $M^+$  383,  $e_p$ : 0°. UV  $\lambda_{max}^{EtOH}$  304 and 259 nm (log  $\epsilon$  3.97 and 4.69 respectively). IR  $\nu_{max}^{Nujol}$  >NH function (3300  $cm^{-1}$ ) typical of a monosubstituted  $\alpha$ ,  $\beta$ -unsaturated amide, 1610  $cm^{-1}$ , a *trans* configuration of the double bond conjugated with amide CO (strong single peak at 1000  $cm^{-1}$ ) and a methylenedioxy group at 920  $cm^{-1}$ . NMR

( $CDCl_3$ )  $\delta$ , 0.90 (*d*, 6H,  $J = 6$ Hz)  $\left( -CH \begin{smallmatrix} \nearrow CH_3 \\ \searrow CH_3 \end{smallmatrix} \right)$ , 1.38 (*s*, 8 methylene protons), 3.15 (*t*, 2H,  $-CO-NH-CH_2-$ ), 2.15 (*m*, 5H allylmethylene and methine protons), 6.70 (*s*, 3H aromatic protons). The secondary amide proton >NH merged with those of the methylenedioxy in a singlet (3H) at 5.90. This data is in quite agreement with that of sylvatine recently reported from *Piper sylvaticum* Roxb. by Banerji *et al.*<sup>1</sup>.

The benzene:ethyl acetate (4:1) and (1:1) eluants gave small quantities of piperine and piperettine only.

We are grateful to Dr. C. V. Reddi Sastry, Synthetic Drugs, Hyderabad, for his interest in this work. Two of us (J. M. R. and K. S.) are thankful to C.S.I.R. for the award of Research Fellowships.

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#### EFFECT OF EXERCISE ON THE TREHALOSE CONTENT OF THE HAEMOLYMPH OF THE DYTISCID BEETLE *CYBISTER CONFUSUS*

In addition to glycogen, it is now well established that the disaccharide trehalose also supports flight muscle activity<sup>1</sup>. Trehalose was identified as the

principal blood sugar in many insect species<sup>2</sup>. As early as 1937 Beutler<sup>3</sup> found that the sugar in blood decreased during flight and, in fact, the duration of flight was limited by the availability of blood sugar. These results therefore suggest that the trehalose is an important metabolite.

It has been reported in our previous investigations that the powerful coxal muscles of the *Cybister* beetle are organized for short rapid actions for which glycogen appears to be the chief fuel source<sup>4-5</sup>. The present investigation is aimed at ascertaining, whether the free sugars especially the trehalose content of the blood is utilized during the induced swimming performance of the *Cybister confusus* beetle.

The beetles employed and the method to induce vigorous swimming activity in them was as described elsewhere<sup>5</sup>.

To collect the haemolymph, the head of the beetle was removed along with the alimentary canal. The cut end of the insect was held against a pre-cooled centrifuge tube. Most of the haemolymph (0.02 ml/beetle) was collected by gentle pressing the abdomen. The body fluid of three to four beetles was pooled and centrifuged for 5 min at 400  $\times$  g at 4°C to remove cells and fat. 5  $\mu$ moles of glutathione/ml blood was added to avoid blackening.

The blood was partially deionized by passing it through Amberlite IR-120 (H-form). Trehalose content of the partially deionized blood was determined by ascending paper chromatography as described by Putman *et al.*<sup>6</sup>, using the solvent system of *n*-butanol:ethanol:water (52:32:16, v/v/v) at room temperature. The standard trehalose was run each time along with the sample. After developing, the paper was cut into two strips, the one containing standard trehalose was treated with ammoniacal silver nitrate to stain trehalose. An area corresponding to standard trehalose of the other strip was macerated in 1 ml distilled water. The trehalose content of the supernatant was determined by anthrone method<sup>7</sup>. The results are expressed as  $\mu$ moles of trehalose per ml blood.

A progressive fall in the trehalose level of the blood during the first 10 min of the exercised beetle is quite evident (Table I and Fig. 1). The concentration of trehalose in the blood of *Cybister* beetle (19  $\mu$ moles/ml blood) appears to be a more important metabolite. The marked decrease in the trehalose concentration of the haemolymph from the onset of exercise for 15 min indicated its utilization by the vigorously working leg muscles. These results thus support the earlier observations that blood trehalose acts as a substrate to supply energy to the working muscles<sup>8-9</sup>. The increase in blood trehalose (Fig. 1) after 15 min of exercise



probably indicates its turnover from the fat body since, the carbohydrates are released into blood mostly as the disaccharide trehalose<sup>7</sup>.

TABLE I

*Trehalose content in the haemolymph of the Cybister confusus during vigorous swimming activity*

Duration of exercise in minutes	Micromoles of trehalose/ml blood		
	Control	Experimental	Rate of reduction
00 (4)	19.00±1.6*	16.70±2.1	2.50
05 (4)	15.50±1.7	11.65±1.6	3.85
10 (4)	18.75±1.2	06.34±0.99	12.41
15 (4)	17.15±1.6	03.10±0.54	14.05
20 (4)	13.50±1.53	06.00±0.80	7.50

The results are averages of four determinations in each case.

\* The results are the mean ± standard error.

The number of insects used in each determination is represented in the parenthesis.

#### TRÉHALOSE CONTENT IN THE HAEMOLYMPH OF THE CYBISTER BEETLE DURING VIGOROUS SWIMMING ACTIVITY

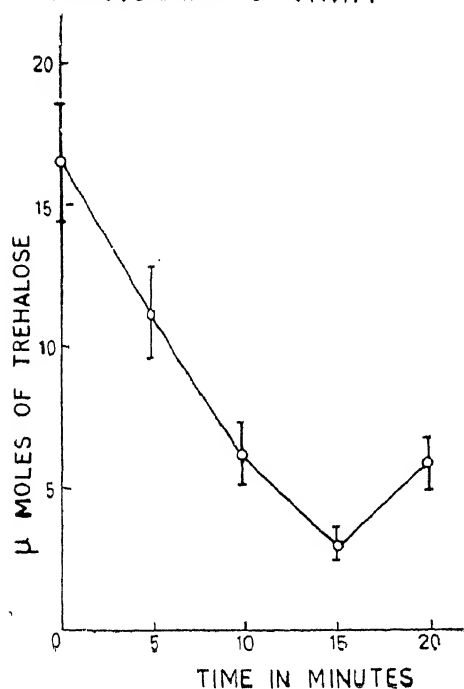


Fig. 1

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#### CONTROL OF HELMINTHOSPORIUM DISEASE OF RICE WITH SOIL AMENDMENTS

HELMINTHOSPORIOSE of rice caused by [*Cochliobolus miyabeanus* (Ito and Kuribayashi) Dresch ex Dastur] *Helminthosporium oryzae* Breda de Hann is generally severe under some soil deficiency conditions, especially when toxic substances accumulate, silica, K, Mn or Mg is deficient or hydrogen sulphide is evolved causing root rot<sup>1-6,11</sup>. Padmanabhan, Abichandani and Patnaik<sup>10</sup> reported that the infection was more in plants under the deficiency of K<sub>2</sub>O. It has also been shown experimentally that under conditions of leaching, a severe incidence of disease could be expected<sup>7</sup>.

Soils from some of the localities in India where the disease had been noticed to be severe, were collected and after analysis, appropriate amendments were tried to see whether the disease intensity could be reduced. The present data deal with soil samples found to be deficient in Mn from Faizabad (U.P.), Ranaghat (West Bengal) and Pattambi (Kerala). The Pattambi soil was not only deficient in Mn but also was highly acidic.

A susceptible variety, Benibhog, was grown in pots filled up with these soils in 2 sets, one without any amendment and a second with the addition of 5, 10 and 20 ppm of Mn. Three seedlings per pot were maintained. The plants were artificially inoculated with spore suspension of 10–12-day old culture of *H. oryzae* when the seedlings were 45 days old. After the artificial inoculation the plants were incubated under humid chambers for 5–7 days,

TABLE I

Infection scores of helminthosporium disease on rice seedlings (Var. Benibhog) grown with addition of Mn in Mn deficient soils

Source of soil	Infection score in Unamended soil	Infection score in amended soil with addition of Mn		
		5 ppm	10 ppm	20 ppm
Faizabad ..	12.20 ± 1.19	5.80 ± 1.29	5.30 ± 1.30	6.20 ± 1.05
Ranaghat ..	10.70 ± 1.25	6.40 ± 1.33	6.30 ± 1.17	7.20 ± 1.37
Pattambi ..	7.45 ± 0.60	5.10 ± 1.40	5.00 ± 0.71	4.40 ± 0.78

C.D. Treat (5%) = 1.56

C.D. Soil (5%) = 1.37

Observations on the disease reaction were taken after 10-12 days of inoculation. The disease was scored as per the score chart developed by Ganguly and Padmanabhan<sup>9</sup>. The data on infection are presented in Table I. The growth of the plants was poor in Pattambi soils as compared to the plants raised in Ranaghat and Faizabad soils.

In Faizabad and Ranaghat soils, the addition of Mn at 5, 10 and 20 ppm as ameliorants reduced the disease significantly but the differences among the different concentrations were not significant, suggesting that the addition of 5 ppm Mn was sufficient to correct the soil. On the other hand in Pattambi soil, the addition of Mn reduced the disease incidence significantly even upto 20 ppm of Mn.

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### SPONTANEOUS CELL SEPARATION AND PROLIFERATION IN *PHOENIX HUMILIS* ROYLE

A NEW phenomenon of cell proliferation in the meristematic tissues of the inflorescence primordia was observed during an investigation of the palm, *Phoenix humilis* Royle. Specimens of this species were collected from plants growing in an estate near Peermedu, Kerala State, at an altitude of 3,500 feet. The actively growing shoots were cut and the leaves removed from the crown until the tender interior of the growing tip was exposed. Later, the young leaf primordia were carefully cut away to reach the shoot apical meristem. The inflorescence primordia were found attached to the base of each leaf primordium. They were extremely flattened and triangular in shape. It was soon found that a fluffy mass of white tissue resembling a fungal growth covered the primordium all along its margin (Fig. 6). Microscopic examination revealed that the constituents of the mass were quite interesting in composition. A detailed study indicated that the whole mass results from proliferation of cells arising from the compact tissues.

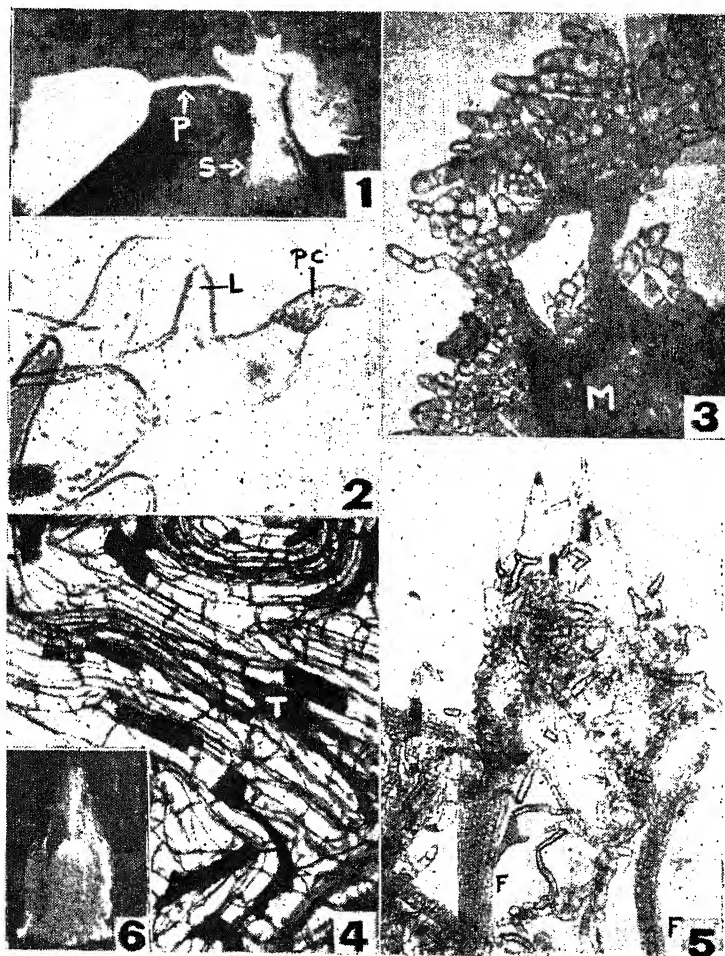
The very young floral primordium measuring 1 mm is a highly flattened triangular structure with a thin margin. The cell proliferation in the margins starts when the primordium is 1.5 mm tall. The cell masses thus produced appear to increase in size until the primordium is 40 mm tall. The proliferating cells together form a loose mass which is conspicuously white when young and turn brownish with age. The overall shape of the mass may be described as 'lanceolate' and its tip grows somewhat longer than the floral primordium (Fig. 6). Internally, the tissue shows many thread-like strands laterally extending from the main body of the primordium. The strands which arise from

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the margins of the primordium put forth branches and turn whitish in colour. The terminal regions of the branches proliferate and result in a mass of cells (Fig. 1). The proliferating cells show a general tendency to separate from each other and grow into filaments some of which become uniseriate while others remain multiseriate to varying degrees (Fig. 5). Many of the terminal cells become completely isolated and remain clumped together in irregular masses (Fig. 5). Some of the

branches divide in the fashion of a filamentous alga (Fig. 3) and yet others resemble various fungal mycelia (Fig. 4).

The early floral primordia exhibit proliferating filaments of short barrel-shaped cells. These filaments often branch dichotomously or trichotomously and the total picture simulates algal forms with heterotrichous habit (Fig. 3). All these filaments could be traced back to strands projecting out as fine threads from the primordium (Fig. 1).



FIGS. 1-6. *Phoenix humilis*. Fig. 1. A transverse slice of the young inflorescence primordium showing a strand of proliferating tissue extending from the margin and branching at the distal end,  $\times 10$ . Fig. 2. Terminal region of a filament showing peg-like lateral growth. The terminal cell contains tanniniferous granules,  $\times 300$ . Fig. 3. Proliferation from a very young primordium showing growth of filaments in an alga-like fashion,  $\times 100$ . Fig. 4. Compact mass of filaments composed of rectangular cells some of them with dark tanniniferous contents,  $\times 100$ . Fig. 5. Filamentous growths showing multiseriate branches and loose proliferation cells accumulating around them,  $\times 80$ . Fig. 6. Isolated floral primordium showing the proliferating tissue around its margin,  $\times 1.5$ .

F—multiseriate filament. L—lateral peg-like growth of cell. M—marginal region of the inflorescence primordium. P—proliferating strand emerging from the margin of the floral primordium. PC—cell containing tanniniferous granules. S—separated cells forming a fluffy mass. T—cell containing dark tanniniferous compounds.

The ends of these filamentous structures when examined under the microscope show extensive separation of cells into uniseriate filaments. With partial separation, the filament itself looks like a multiserial algal structure (Fig. 5). The proliferating cells are highly enlarged, widely separated and resemble cells of a loose callus. Short filaments of three or four cells attached by short distances of their common cell walls were also found. Other interesting patterns of cells were also observed in the filamentous mass. Some of the subterminal cells of the uniseriate filaments put forth lateral projections which are subsequently cut off as new cells which may start new filaments. Such peg-like lateral growths also occur in the cells occupying the middle regions of the filaments (Fig. 2). Another feature of interest was that isolated cells in the mass showed thickening of their walls and accumulation of tanniferous compounds and stand out as dark cells in a mass of transparent ones. One more peculiarity observed in the terminal cells was the accumulation of tanniferous granules which sometimes filled the entire cell (Fig. 2). Some of the proliferating cells elongate into rectangular structures and form loose fitting filaments. At one stage of their proliferation they look like mats of fungal hyphae. Moreover, the filaments also break into shorter ones and the mass becomes fluffy. Dark tanniferous compounds accumulate in these cells also.

It is of interest to note that this complex mass of cells arising from the margin of the floral primordia is cast off at later stages and this completely obscures the origin of the fluffy mass. A similar growth is also seen to some extent in the margins of the developing leaf primordia as well as in the corners of the plications. Further, schizogamous separation of the meristematic cells of the leaf is also known (Padmanabhan, 1967).

In the taxonomic literature, the term 'wool' is used to describe such masses of cells which are usually considered to be composed of epidermal hairs and scales together called as indumentum. In several palms, epidermal hairs of the foliar lamina show irregular forms indicating a tendency of the cells to proliferate forming filaments and scales (see Tomlinson, 1961). But the proliferating masses described in this contribution are quite different and relate to very early stages of development while the foliar hairs are of late origin. Furthermore, it is doubtful whether the proliferation of the kind occurs in any other part of the palm. The epidermal structures of the palms need better elucidation and detailed study in view of the present findings.

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#### STOMATAL REGULATION AND SUBSIDIARY CELLS IN *CROTALARIA MEDICAGINEA* LAMK.

THE stomatal apertures are influenced by a number of environmental factors both external and internal. It is generally agreed that stomatal movement is brought about by the changes in the turgor of the guard cells<sup>1</sup>, and of the neighbouring epidermal cells<sup>2</sup>. The role of starch  $\rightleftharpoons$  sugar regulation in the guard cells has been considered to be an important cause of stomatal opening<sup>3</sup>, caused by the influx of potassium ions<sup>1,7,8,10</sup>. Plasmolysis of the epidermal cell has also been shown to be the cause of stomatal opening<sup>2</sup>. It has been shown that ions are taken up directly from the solution and accumulate in guard cells by an active uptake mechanism in the detached epidermis in respect to certain solution in medium<sup>3,4</sup>. It was found difficult by some<sup>11</sup> in accepting Fujino's<sup>4</sup> further suggestion that normal opening in light, in an intact leaf, is a result of active cation transport into the guard cells from the other leaf cells, including those of mesophyll. When stomates of *Zea mays* opened, K and Cl migrated from the subsidiary cells into the guard cells; when they closed both elements returned to the subsidiary cells<sup>8</sup>.

During the course of investigations of water relations of some desert plants, interesting cases of stomatal regulation with possible uptake of cations from the incubating media and the role of subsidiary and normal epidermal cells were observed. The same is reported here on isolated epidermal peelings of *C. medicaginea*.

Epidermal peelings from the leaves of *C. medicaginea* were incubated in different concentrations (0.4–1.0 M) of KCl and NaCl for a period of 1–3 hours and their controls in distilled water. The preliminary trials with 0.1 to 3.0 M of these chemicals were first made before detailed experi-

ments. After incubation period, the epidermal peelings were stained with neutral red to observe the plasmolysis, if any. Stomatal pore width was measured with a precalibrated microscope. It was interesting to observe that there existed two distinct types of stomata in this species: (i) those having only one subsidiary cell which became distinct as it was stained red with neutral red together with guard cells, and (ii) those having no subsidiary cell and stomata were surrounded by ordinary epidermal cells. The openings in these two types of stomata were almost always different from one another. The epidermal peelings were also tested for the presence of starch in guard cells with iodine. The measurements for stomatal apertures in different concentrations of KCl and NaCl with plasmolysis, if any, are shown in Table I.

It appears from Table I that stomatal openings in the second type of stomata were always more or less twice as compared to the first type. Only subsidiary and guard cells were stained with neutral red, which appeared to have high absorptive capacity for the solutes in the incubating media. Plasmolysis was remarkably distinct only in the subsidiary cells, but this did not bring about any increase in the stomatal pore width. Only when guard cells plasmolysed, stomatal pore got reduced. It was also evident that stomata opened to their maximum somewhere at 0.4 M KCl and 0.6 M NaCl due to the possible transport of ions into the guard cells from the incubating media. If plasmolysis has any role, it is through the sub-

diary cells, because epidermal cells are not stained distinctly with neutral red. It is evident that guard cells must absorb cations directly if there are no subsidiary cells; with the result the stomata open quicker and much wider as compared to those having subsidiary cells, as the absorption of ions in the latter is *via* subsidiary cells (Fig. 1).

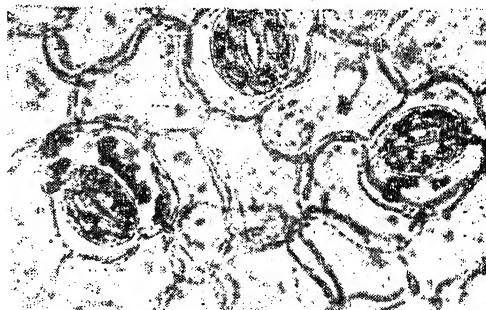


FIG. 1. Stomata without subsidiary cell (middle) more open than those with subsidiary cell (right and left) in *C. medicaginea*.

A remarkably distinct particulate movement in subsidiary cells, together with globular bodies at times were observed, which might be having a role in the movements of cations and stomatal regulation.

Further work is in progress.

The authors are grateful to Prof. Dr. H. C. Arya for facilities and to Dr. D. D. Chawan and Shri K. G. Ramawat for help in various ways.

TABLE I

Effect of an incubation period of 1 hour in different concentrations of KCl and NaCl on stomatal pore width (in  $\mu$ ) and plasmolysis of subsidiary cells in isolated epidermal peelings of *Crotalaria medicaginea*

Conc. in Mol.	KCl			NaCl		
	Stomata with subs. cell	Stomata without subs. cell	Plasmolysis	Stomata with subs. cell	Stomata without subs. cell	Plasmolysis
0	1.3 $\pm$ 0.2	1.8 $\pm$ 0.5	—	1.3 $\pm$ 0.2	1.8 $\pm$ 0.5	—
0.4	4.5 $\pm$ 0.9	9.6 $\pm$ 1.0	—	2.2 $\pm$ 0.7	6.1 $\pm$ 0.8	—
0.5	4.0 $\pm$ 0.9	9.3 $\pm$ 0.6	—	3.4 $\pm$ 0.7	6.4 $\pm$ 0.7	—
0.6	3.7 $\pm$ 1.0	7.6 $\pm$ 0.6	—	3.7 $\pm$ 0.7	6.6 $\pm$ 0.7	—
0.7	3.1 $\pm$ 0.4	6.1 $\pm$ 0.8	+	2.4 $\pm$ 0.7	3.9 $\pm$ 0.7	+
0.8	2.2 $\pm$ 1.8	5.4 $\pm$ 1.0	+	0.9 $\pm$ 0.7	2.7 $\pm$ 0.6	+
0.9	1.0 $\pm$ 0.7	3.9 $\pm$ 0.7	+	0.3 $\pm$ 0.6	2.4 $\pm$ 0.7	+
1.0	0.4 $\pm$ 0.7	3.4 $\pm$ 0.7	+	0.3 $\pm$ 0.6	2.4 $\pm$ 0.7	+

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### BIOSTRATIGRAPHIC ZONES IN THE ARCHIPELAGO GROUP OF THE NEILL ISLAND, SOUTH ANDAMAN

AN attempt has been made for the first time to delineate a Partial-range zone, and two Assemblage-zones in the Late Miocene-late Pleistocene rocks, exposed in the Neill island. Singh *et al.*<sup>1,2</sup> recorded the occurrence of the various microfauna from these beds. The following geological sequence of the beds was recorded in the Neill island by Singh *et al.*<sup>2</sup>.

		Approximate thickness in metres
Archipelago Group	Coastal coral beds, beach sands and soil	Recent 1-3
	----- Unconformity -----	
	Neill Island Limestone	Late Pleistocene 1-4
	----- Unconformity -----	
	Western Coast Limestone	Pliocene 20
	Eastern Coast Mudstone	Late Miocene-early Pliocene 155
	----- Base not exposed -----	

The following biostratigraphic zones have been demarcated in the ascending order in the Archipelago Group exposed in the Neill Island :

- (i) *Globorotalia* (*G.*) *tumida*—*tumida*—*Sphaeroidinellopsis subdehiscens panedehiscens* Partial-range zone (late Miocene-early Pliocene; late Messinian-early Zanclean); Zone N. 18, Blow, 1967<sup>3</sup> : The present zone is recorded in the Eastern Coast Mudstone which is very well exposed on the eastern coast of the Neill Island. The zone is recognized by the presence of a rich assemblage of planktonic foraminifera

which are as follows : *Globigerina* sp., *G. bulloides apertura* Cushman, *G. bulloides bulloides* d'Orbigny, *G. nepenthes* Todd, *G. ouachitaensis ciproensis forma atypica* Bolli, *G. praebulloides praebulloides* Blow, *G. juvenilis* Bolli, *Globoquadrina altispira altispira* (Cushman and Jarvis), *G. dehiscens dehiscens* (Chapman, Parr, and Collins), *G. larmeri obesa* Akers, *Globorotalia* spp., *G. crassula crassula* Cushman and Stewart, *G. cultrata cultrata* (d'Orbigny), *G. acostaensis acostaensis* Blow, *G. cultrata ? limbata* (Forrasini), *G. margaritae* Bolli and Bermúdez, *G. merotumida* Blow and Banner, *G. tumida plesiotumida* Blow and Banner, *G. multicamerata* Cushman and Jarvis, *G. tumida tumida* Brady, *Turborotalia obesa* Bolli, *Hastigerina aequilateralis aequilateralis* (Brady), *H. aequilateralis involuta* (Cushman), *Pulleniatina* sp., *Sphaeroidinellopsis subdehiscens panedehiscens* Blow, *S. subdehiscens subdehiscens* (Blow), *S. seminulina seminulina* (Schwager), *S. seminulina kochi* (Caudri), *Globigerinoides quadrilobatus altiapertura* Bolli, *G. bollii* Blow, *G. quadrilobatus quadrilobatus* (d'Orbigny), *G. quadrilobatus immaturus* Le Roy, *G. obliquus ? extremus* Bolli, *G. obliquus obliquus* Bolli, *G. ruber* d'Orbigny, *G. quadrilobatus sacculifer* (Brady), *G. quadrilobatus trilobus*

(Reuss), *Globigerinoides* spp. and *Orbulina universa* d'Orbigny.

The present zone is conformably overlain by the *Pulleniatina obliquiloculata praecursor*—*Globigerinoides ruber* Assemblage-zone.

- (ii) *Pulleniatina obliquiloculata praecursor*—*Globigerinoides ruber* Assemblage-zone :  
Typical section : Western coast of the Neill Island.

Litho-unit : Western coast Limestone.

Description : The zone is named after the planktonic foraminiferal species *Pulleniatina obliquiloculata praecursor* (Parker and

Jones) and *Globigerinoides ruber* d'Orbigny which occur commonly. The other characteristic associated planktonic foraminifera are *Globigerinoides quadrilobatus immaturus* Le Roy, *G. quadrilobatus trilobus* (Reuss), *Globoquadrina larmeni obesa* Akers and *Turborotalia obesa* Bolli. The present assemblage of the planktonic foraminifera suggests a Pliocene age to this Zone and it may be correlated with the zone N. 19, *Sphaeroidinella dehiscens dehiscens*—*Globoquadrina altispira altispira* Partial-range zone (emended ex Banner and Blow, 1965<sup>1</sup>, Blow, 1967<sup>2</sup>), Pliocene.

- (iii) *Globigerina bulloides bulloides*—*Globorotalia cultrata cultrata* Assemblage—zone.

*Typical section*: Central part of the Neill Island.

*Litho-unit*: Neill Island Limestone.

*Description*: The present Assemblage-zone lies unconformably over the *Pulleniatina obliquiloculata praecursor*—*Globigerinoides ruber* Assemblage-zone and contains a few planktonic foraminifera—*Globigerina bulloides bulloides* d'Orbigny, *Globorotalia cultrata cultrata* (d'Orbigny) and *Globigerinoides* sp. However, the zone has yielded a rich assemblage of benthonic foraminifera and it may be referred to late Pleistocene on account of its stratigraphic position.

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#### A POSSIBLE VIRUS MALADY OF RICE

A RICE disease was spotted out in experimental plot at Irrigation Research Centre, Chakuli, in the district of Sambalpur, Orissa, during the month of September, 1972. An early maturing high yielding dwarf variety, Bala, was affected with the disease. Later observations revealed that the malady was

also prevalent in hilly tracts of the district in local *indica* rice varieties.

Affected plants are stunted with profuse tillers. Such diseased plant populations were found in large numbers towards the periphery of plot. Numerous slender diminutive tillers are produced giving the plant a rosette appearance. The leaves are short, narrow, bluish-green in colour, with erect habit. Very fine inconspicuous longitudinal chlorotic streaks are observed in basal portion of leaves parallel to midrib. However, such streaks are not commonly present in newly emerging leaves. Affected plants remain green until harvest. The most characteristic symptoms of the disease are the production of short, compact, stout, earhead very much resembling like that of a "Bajra panicle" (Fig. 1). The grains are short, shrivelled and discoloured. The heads also show partial emergence from the leaf sheath.

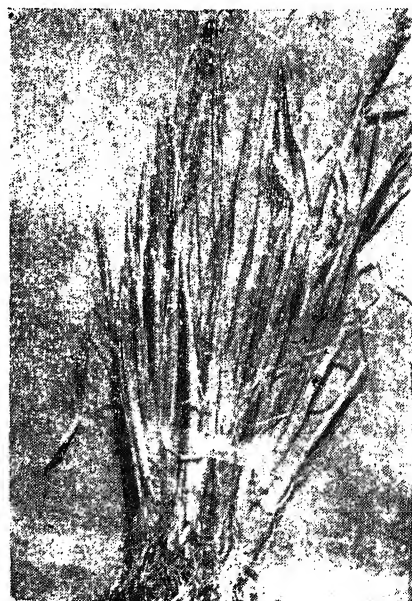


FIG. 1. Affected rice plant showing excessive tillers and "Bajra-like panicle".

The diseased plot was found naturally infested with insects, namely, *Nephotettix impicticeps*, *N. apicalis* (Motsch.), *Sogatella furcifera* (Horv.), *Recilia dorsalis* (Motsch.), and *Nilaparvata lugens* (Stal.). The population of *N. lugens* was 4 per sweep only. Transmission study was undertaken by using single plant caging technique (Everette, 1969) in glass chimney. Fourteen day old Taichung Native I seedlings were used as test plants. Each cage was inoculated with three leaf hoppers of one species collected from the diseased field at a time in the early morning hours. Two control series

were maintained—one with no insect, the other with three non-viruliferous insect, but caged. Tests were repeated thrice. Plants, inoculated with the vector *N. lugens* only, showed symptoms 10–15 days after inoculation. On an average 33.3% of plants got infected. Seed transmission also was tried but found negative. The major symptomatology, and vector specificity the disease approximately agrees with Grassy stunt of rice reported by Rivera *et al.* (1966). However, the panicle symptoms poses a doubt, since it has not yet been described elsewhere. (Attempt was made to isolate any kind of plant pathogenic fungi or nematodes from the diseased plants, but without any success.) This may be an additional new symptom which is expressed in certain environmental conditions.

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#### CIRCADIAN PERIODICITY IN THE INCIDENCE OF AIR-BORNE SPORES OF *SPORORMIA*, *PITHOMYCES* AND *SPGAZZINIA*

THE Hirst spore trap data, collected at a height of 1.22 m above ground level during 1966–67 in a sugarcane plot at Anakapalle, concerning the incidence of the spores of *Sporormia* type, *Pithomyces* type and *Spegazzinia deightonii* in the air for 24-hour periods are presented in this paper. Figure 1 gives the mean circadian periodicity curves for the three spore types; the curves are constructed from the estimated percentages of the peak arithmetic mean concentration.

*Sporormia* type occurred during day time between 08.00 and 18.00 hr with higher concentrations in the period 10.00–12.00 hr. The estimated peak mean concentration was 18/m<sup>3</sup> of air; the

highest catch being 147/m<sup>3</sup> of air on 29 November 1966 at 12.00 hr.

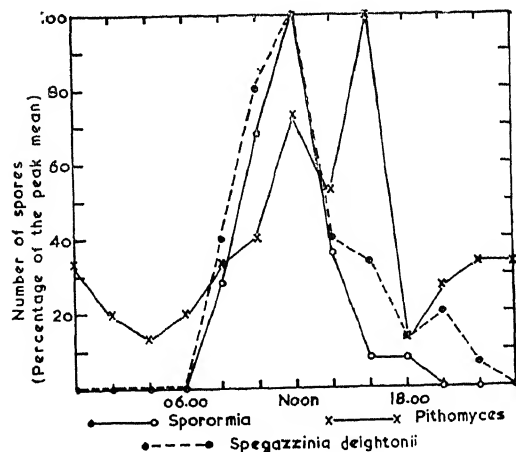


FIG. 1. Mean circadian periodicity curves of *Sporormia* type, *Pithomyces* type and *Spegazzinia deightonii* based on the data for 30, 49, and 40 days respectively. The curves are expressed as a percentage of the peak arithmetic mean concentration.

*Pithomyces* type was seen intermittently throughout 24-hour period; relatively higher numbers were encountered in the period 12.00–16.00 hr. The estimated peak mean incidence was 6/m<sup>3</sup> of air; the maximal incidence being 105/m<sup>3</sup> of air at 16.00 hr on 24 March 1967.

*Spegazzinia deightonii* appeared between 08.00 and 22.00 hr peaking at 12.00 hr. The estimated peak mean number was 8/m<sup>3</sup> of air; the highest incidence being 42/m<sup>3</sup> of air on 1 and 26 December 1966 and on 11 January 1967 at 10.00 hr.

It is discernible from Fig. 1 that the rise from a low concentration is steep and the subsequent decrease is relatively slow with *Sporormia* and *Spegazzinia deightonii*, while with *Pithomyces* the reverse is the case.

The behaviour of *Sporormia* with its mid-day peak is in variance with that of many ascospore types which usually occur in air during night time; it is in agreement with the findings of Meredith<sup>2</sup> and Ingold<sup>1</sup>. The periodicity pattern of *Pithomyces* agrees with the report of Meredith<sup>2</sup>. The circadian periodicity of *Spegazzinia deightonii* differs from that described for *Spegazzinia* (*S. sundara* and *S. tessarthra*, both counted together) by Sreeramulu and Ramalingam<sup>3</sup> who obtained two subsidiary peaks, one in the early morning and another in the forenoon in addition to the main peak at 20.00 hr. Probably different species of the same genus show different peaks.



The circadian periodicity described for the three fungal spore types forms the first report from India.

Thanks are due to Prof. T. Sreeramulu for advice and permission to the use of Hirst-trap slides.

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#### LEVELS OF ENDOGENOUS GROWTH SUBSTANCES IN SWEET ORANGE LEAVES AFFECTED BY MOSAIC VIRUS

MOSAIC disease on sweet orange trees has been recently reported from Citrus growing tracts of Andhra Pradesh<sup>1</sup>. The symptoms are the irregular yellow or light green patches alternating with normal green leaf area distributed all over the leaf in the initial stages<sup>1</sup>, ultimately leading to complete yellowing and leaf fall. No detailed investigations seem to have been carried out on the levels and changes of endogenous growth substances in virus affected Citrus plants except for a few studies on exocortis<sup>2</sup>. Therefore, an attempt has been made in the present study to estimate the endogenous growth substance levels in healthy and mosaic infected sweet orange (Sathgudi) leaves.

One year old Sathgudi nucellar seedlings were inoculated with mosaic virus in November 1972. Leaves showing severe symptoms were collected in July 1973. Leaves from healthy plants of the same age kept as check were also analysed for comparison. The leaf number from apex was maintained constant for both healthy and diseased so as to keep the age factor constant. The methanolic extract of the leaves (equivalent to 3 gm fresh weight) was re-extracted with diethyl ether according to the method of Goldschmidt and Monselise<sup>3</sup> and the developed chromatograms in the solvent mixture (Isopropanol: ammonia: water—80.0 : 0.1 : 19.9) were cut into ten equal segments and the bioassay was carried out according to the method of Das *et al.*<sup>4</sup>. The results presented in the histograms (Fig. 1) are the means of four independent estimations in both the healthy and infected leaves. The data were statistically analysed.

It could be seen from the histograms (Fig. 1) that, in general, growth promoting activity was

clearly evident in the healthy leaves (Fig. 1 A) while it was totally absent in the diseased (Fig. 1 B). The growth promotion at  $R_f$  0.2–0.3 corresponding to the IAA zone was statistically significant, apparently indicating the presence of IAA in Citrus tissues in conformity with the observations of Goldschmidt *et al.*<sup>5</sup>. The inhibitory activity in the healthy leaves was significant at  $R_f$ s 0.4–0.5, 0.7–0.8 and 0.9–1.0. While the inhibitory activity at  $R_f$  0.4–0.5 could be due to the presence of  $\beta$ -inhibitor, the same at the remaining  $R_f$ s could not be accounted for. In the virus infected leaves, on the other hand, significant inhibitory activity was observed at all the  $R_f$ s (Fig. 1 B) and it was highly significant at  $R_f$  0.6–0.7. The presence of

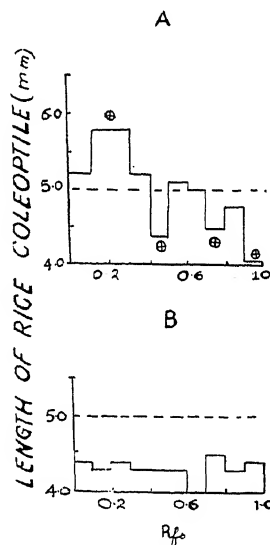


FIG. 1. Endogenous growth substances in (A) healthy and (B) diseased Sweet Orange leaves.

Note: Dotted lines indicate the rice coleoptile growth in sucrose control. '⊕' in (A) indicates significance at 1% level.

significant inhibitory activity at  $\beta$ -complex region (0.4–0.7) of which abscisic acid (ABA) was found to be a major constituent by Milborrow<sup>6</sup> apparently suggests the presence of ABA in virus infected Citrus leaves. In exocortis infected Citrus terminals Hanks and Feldman<sup>2</sup> also observed a lowering of promoting activity accompanied by an enhancement in the inhibitory activity. Thus mosaic virus infection seems to alter the promoter/inhibitor balance totally in favour of inhibitor. The first author is thankful to the C.S.I.R. for the financial assistance.

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#### COLCHICINE FROM THE SEEDS OF *GLORIOSA SUPERBA*

COLCHICINE, an important alkaloid used in the treatment of gout and in plant breeding work for inducing polyploidy, is at present, extracted from the corms of *Colchicum autumnale*, occurring wild in some parts of Europe. Recently, there had been an increase in the demand of this alkaloid but the supplies from the conventional sources had not been sufficient to cope with it. A worldwide search for an alternative plant source is being carried out but no suitable raw material has yet been found. Among the Indian plants the corms of *Colchicum luteum* and the seeds of *Iphigenia stellata* containing 0.25% and 0.9% of colchicine respectively<sup>1,2</sup> are not available in sufficient quantities to warrant any commercial utilization. *Gloriosa superba* is another plant containing colchicine. Chemical evaluation of a number of tuber samples, however, indicated a very low alkaloid content. On the other hand the ripe seeds of the plant, analysed according to Santavy<sup>3</sup>, gave a yield of 0.81% of total alkaloids (d.w.b.) and 0.60% of colchicine (d.w.b.). The tubers of the plants from which the seeds were obtained, on similar analysis, yielded 0.57% of total alkaloids and only 0.05% of colchicine. It is evident that the percentage of colchicine in the seeds is more than ten times of that in the tubers.

*Gloriosa superba* Linn. (Fam.: Liliaceae) is a perennial herbaceous climber, occurring among scrub forests throughout India upto an altitude of 1800 m. It flowers during the months of July and August and the ripe seeds, borne in capsules 5 to 6 cm long, can be harvested towards the end of September. The plant is easily propagated by tuber cuttings. The tuber cuttings, planted in the month of May, sprout in July and the fruits with

ripe seeds are ready for harvest in the first week of October. The plant being a perennial, the fruits can be harvested for a number of years from the same planting, thus ensuring a sustained supply of the raw material to the industry. The high colchicine content accompanied by prospects of good availability from both wild and cultivated sources make the seeds of *Gloriosa superba* a potential commercial source of colchicine in India. Resources survey and cultivation trials have since been taken up for the development of this raw material.

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#### THE ENDOSPERM ORGANIZATION IN *MICROCARPAEA* R. Br. (SCROPHULARIACEAE)

THE embryology of several genera belonging to the Scrophulariaceae has been examined<sup>1</sup>. The genus *Microcarpaea*, however, appears to have remained uninvestigated. The present note deals with the development of endosperm in *Microcarpaea muscosa* R. Br. The material for study was collected from the river Cauvery near Thalakadu, Mysore District.

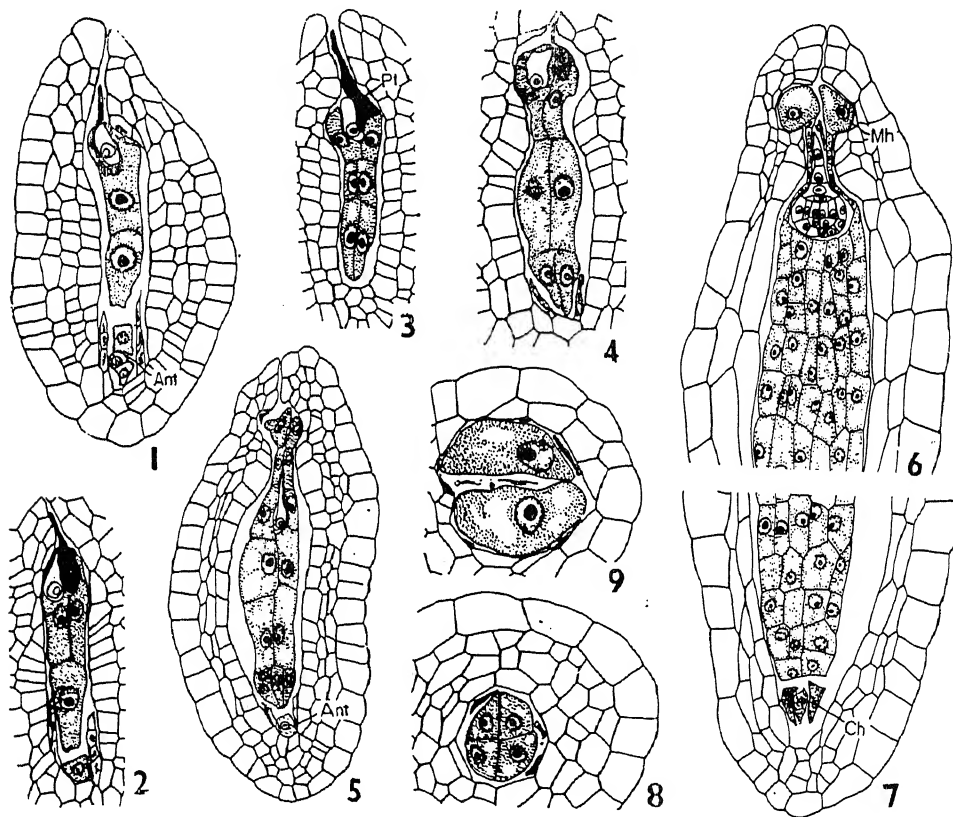
The development of the female gametophyte in the anatropous, tenuinucellate and unitegmic ovule conforms to the Polygonum type. Fertilization is porogamous. The pollen tube enters the embryo sac destroying one of the synergids and liberates the two male gametes. Syngamy and triple fusion have been observed. The surviving synergid breaks down soon after double-fertilization. The antipodal cells persist within the confines of the nucellus for some time, in several cases.

The first division of the primary endosperm nucleus is followed by the laying down of a transverse wall. Of the two resulting primary chambers the chalazal one is shorter (Fig. 1). The next division is vertical and it occurs in both chambers. Thus two tiers of two cells each are formed (Fig. 2). The cells of the chalazal tier undergo vertical division to form a tier of four cells, which function together as the chalazal haustorium (Fig. 5). These cells generally acquire dense cytoplasm and their activity ceases during the later part of seed development. The cells do not fuse together and ultimately degenerate as such (Fig. 7, 8).

The two juxtaposed cells derived from the primary micropylar chamber divide transversely and produce two superposed tiers of two cells each (Fig. 3). The upper tier directly functions as the micropylar haustorium and the lower acts as the initial of the endosperm proper. The two cells of the micropylar haustorium enlarge, crushing the surrounding cells (Figs. 6, 9). They neither fuse

development the integumentary tapetum is crushed and absorbed (Figs. 4-7). The mature seed is albuminous.

The mode of organization of endosperm in *Microcarpaea muscosa* is essentially similar to that of *Mimulus ringens*<sup>2</sup>, a closely allied member, with the difference that in the latter the cells of the micropylar haustorium ultimately become binucleate.



FIGS. 1-9. Endosperm of *Microcarpaea muscosa*. (Ant, antipodal cells; Ch, chalazal haustorium; Mh, micropylar haustorium. Pt, pollen tube.) Fig. 1. Two-celled endosperm in longitudinal section of a very young seed,  $\times 560$ . Fig. 2. Four-celled endosperm,  $\times 560$ . Fig. 3. Six-celled endosperm,  $\times 560$ . Fig. 4. Division in the initials of endosperm proper,  $\times 560$ . Fig. 5. L.s. young seed showing two-celled and four-celled micropylar and chalazal endosperm haustoria respectively,  $\times 375$ . Figs. 6-7. L.s. micropylar and chalazal parts of the same seed showing endosperm tissue and embryo,  $\times 375$ . Fig. 8. Part of t.s. of seed through the 4-celled chalazal haustorium,  $\times 560$ . Fig. 9. Part of t.s. of seed through the micropylar haustorium showing the two uninucleate cells,  $\times 560$ .

together nor develop lateral extensions. Their degeneration occurs subsequent to that of the chalazal haustorium.

The two cells of the endosperm proper undergo a series of transverse and longitudinal divisions and produce a massive endosperm tissue which nourishes the embryo. During later stages of endosperm

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TABLE II

Total protein, nitrogen, amino acid and phosphorus (expressed as mg/100 mg) of healthy and Yellow vein infected Bhindi fruit

Age of the fruits in days	Protein	Nitrogen	Amino acid	Phosphorus		
4 Healthy ..	7.6887	2.3544	1.780	.1900		
Diseased ..	13.5212	2.4402	1.540	.1966		
7 Healthy ..	6.2551	2.5452	1.410	.1800		
Diseased ..	10.5882	2.5452	1.160	.1966		
12 Healthy ..	5.3025	2.2694	0.620	.1700		
Diseased ..	9.0137	1.8876	0.220	.1600		
F calculated value					F tabulated value	
Infection ..	53.91*	0.18	33.00*	0.15	1% 98.49	5% 18.51
Age ..	10.23	1.40	202.20**	4.80	99.01	19.00

\* Significant at 5%, \*\* Significant at 1%.

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#### OCCURRENCE OF DIMORPHIC EMBRYO SACS IN *TRICHOSANTHES LOBATA* ROXB.

*Trichosanthes lobata* Roxb. is a member of the sub-tribe Trichosantheae of the tribe Trichosantheae of the economically important family Cucurbitaceae. The development of embryo sac is monosporic Polygonum type<sup>1-5</sup>, but few interesting variations are reported in recent years. Chopra and Agrawal<sup>6</sup> and Dzevaltovsky<sup>7</sup> have described usually bisporic and rarely monosporic embryo sacs in *Benincasa*

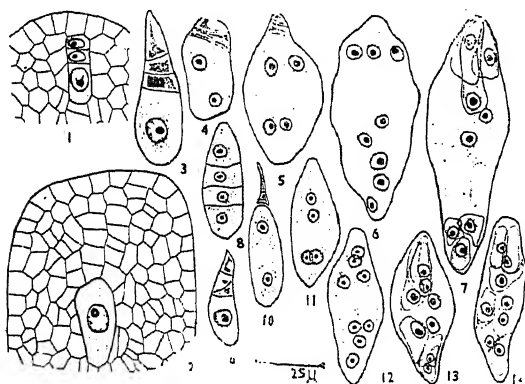
*cerifera* while Dzevaltovsky<sup>8</sup> has reported the development of embryo sacs directly from the female archesporial cell in *Momordica charantia*. Singh and Dathan<sup>9</sup> more recently observed somatic apospory in *Cucumis metuliferus*. Generally in Angiosperms the embryo sacs in a species are fairly constant in their size. While studying the embryology and seed development of cucurbitaceous members, the author came across large and small embryo sacs in one and the same ovary of *Trichosanthes lobata* Roxb., collected from Katihar, Bihar State, and the same is reported in this communication.

The hairy ovary is inferior, tricarpeal and unilocular with three parietal placentae, each of which is bifid and T-shaped bearing a single ovule on each flank. The ovules are anatropous, bitegmic and crassinucellate and the vascular supply passes through the outer integument upto the tip of the ovule as described for other members of Cucurbitaceae. The earliest stage in megasporogenesis observed is the young megaspore mother cell capped by two parietal cells (Fig. 1). By further periclinal and anticlinal divisions in the parietal cells, the megaspore mother cell becomes deep-seated (Fig. 2). Further development in megaspore mother cell results in the occurrence of dimorphic monosporic Polygonum type of embryo sacs and all the intermediate stages of development of large (Figs. 3-7) and small (Figs. 8-14) embryo sacs show difference in their size.

The megaspore mother cell enlarges and undergoes meiosis forming a linear tetrad of megaspores

(Figs. 3, 8, 9). The functional megaspore is chalazal while the three remaining degenerate (Figs. 3-5, 9, 10). The nucleus of the functional megaspore undergoes three divisions forming 2-, 4- and 8-nucleate embryo sacs (Figs. 4-6, 10-12). In the organised embryo sac, the antipodals are also large in size and the egg is conspicuously larger than the synergids.

Even though, dimorphism is exhibited at the megaspore stage itself, it is now conspicuous when the embryo sac is fully organised (Figs. 3, 9, and 7, 13, 14). At this stage, in about 20% cases, the embryo sacs remained small and component cells almost filled it completely giving it a cellular appearance (Figs. 13, 14). The large embryo sacs usually



FIGS. 1-14. Development of dimorphic embryo sacs in *Trichosanthes lobata*. Figs. 1-7. Stages in the development of large embryo sac. Figs. 8-14. Same, of small embryo sac.

attain an average size of  $100 \mu \times 30 \mu$  while the smaller ones are  $60 \mu \times 20 \mu$  in size. Both types of embryo sacs are observed in the same ovary and even on either side of the same T-shaped placenta. The cause for the arrested growth of embryo sacs is not known, but after fertilization, both these ovules are observed to develop into normal seeds.

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### ROLE OF CATIONS AND EPIDERMAL CELL TURGOR IN STOMATAL OPENING OF *CYNANCHUM PAUCIFLORUM* R. Br.

A LARGE number of theories have been proposed by various workers on the role of cations and epidermal cell turgor in stomatal opening<sup>1-4</sup>. Besides  $K^+$  many other cations (Na, Ca, Rb, Sr, Fe, etc.) cause the opening of stomata by their active uptake. Out of these  $K^+$  have been stated to be most actively absorbed as it is evident from a number of published papers. Stomatal movement is also brought about by a change in the turgor of the guard cells<sup>3</sup> and neighbouring epidermal cells<sup>5-6</sup> and that these changes may be caused by osmotic gradients between guard cells and neighbouring tissue. The present work was carried out in order to have further information with regard to uptake of certain cations and the role of epidermal cell turgor in isolated epidermal peelings of *C. pauciflorum*.

Epidermal peelings from the leaves of *C. pauciflorum* were incubated in respective Molar (0.1-1.0) solutions of KCl, NaCl, BaCl<sub>2</sub>, MgCl<sub>2</sub> and sucrose for a period of 6 hours in continuous light (about 1000 lux). After the incubation period, stomatal pore width, plasmolysis if any, starch in the guard cells were observed as described earlier<sup>7</sup>.

All concentrations of KCl were effective in causing the opening of stomata Fig. 1. The maximum stomatal opening was observed in 0.5 M solution where nearly cent per cent epidermal cells were plasmolysed. The lower concentrations of NaCl, BaCl<sub>2</sub>, MgCl<sub>2</sub> remained ineffective although slight opening was caused in higher concentrations except for MgCl<sub>2</sub> which was completely ineffective in causing any opening.

When peelings were incubated in a mixture of all these four salts (NaCl, KCl, BaCl<sub>2</sub> and MgCl<sub>2</sub>) in equal proportions, stomata opened. When KCl was deleted from this mixture stomatal opening was caused but width was lesser than when KCl was included. With the deletion of NaCl from the mixture, stomata opened still lesser, proving thereby that  $K^+$  and Na were mainly effective in

causing stomatal opening in this species. The effect of the mixtures on the stomatal pore width observed was as follows :

(Concentration 0.5M and ratio 1:1:1:)

KCl + NaCl + BaCl<sub>2</sub> + MgCl<sub>2</sub> = 9.6  
NaCl + BaCl<sub>2</sub> + MgCl<sub>2</sub> = 5.3  
BaCl<sub>2</sub> + MgCl<sub>2</sub> = 3.0  
Water = Close

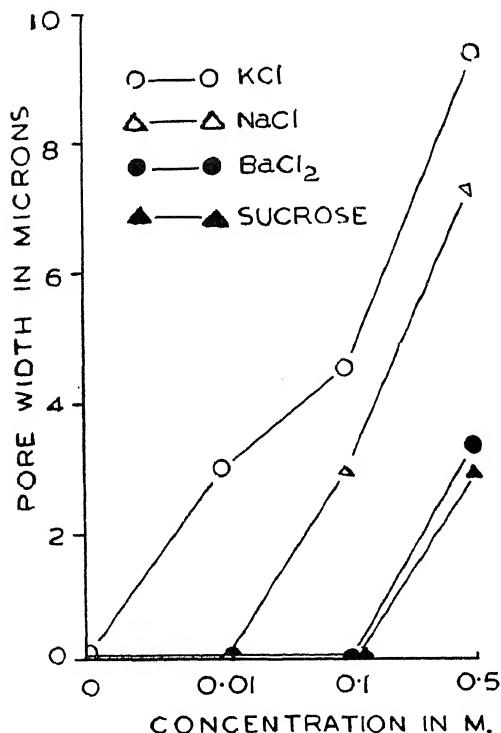


FIG. 1. Effect of KCl, NaCl, BaCl<sub>2</sub>, and sucrose in different concentrations on the stomatal pore width in isolated epidermal peelings of *C. pauciflorum*.

The increase in the osmotic pressure of the guard cell in the presence of KCl was the primary cause of stomatal opening which probably was caused by the reduction in epidermal cell turgor<sup>6,8</sup>. In *C. pauciflorum*, KCl caused maximum opening with plasmolysis in surrounding epidermal cells. Stomata also opened in this species when incubated in sucrose solution where also plasmolysis in epidermal cells was observed. If the reduction in turgor of epidermal cells is the main cause of stomatal opening, then the stomata incubated in MgCl<sub>2</sub> must also open as plasmolysis of epidermal cells is caused by this salt also. Thus the main uptake was that of K<sup>+</sup> followed by Na<sup>+</sup> and Ba<sup>++</sup>, which were also responsible for stomatal opening

but not the Mg<sup>++</sup>. Starch in the guard cells reduced very much during uptake of certain cations causing stomatal opening and reduction in the turgor of the epidermal cells. The higher concentration of K<sup>+</sup> in the guard cells was related to starch hydrolysis which increased the osmotic potential, resulting in their uptake of water and opening of stomata<sup>8,9,10</sup>. Thus, it is concluded that hydrolysis of starch on uptake of certain cations, and plasmolysis of the epidermal cells reducing the turgor potential are the factors responsible for the stomatal opening.

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#### ALTERNARIA LEAF SPOT OF SUNFLOWER

A SEVERE leaf spot disease of sunflower was observed in November-December 1972, around Bangalore, India. In two plots, the disease was so severe that the plants died prematurely. The disease symptoms first appear as yellow spots on leaves which later turn to brown spots with serrated margin. In severe cases, the spots coalesce killing a larger area. As the disease advances, spots appear on petiole, stem, petals and sepals. The spots on the stem and petiole are linear with a halo spot in the centre. The spots on sepals will be irregular to round with a halo centre (Fig. 1). In severe cases, seeds also get infected. The disease

is very severe in later stages, although plant is susceptible at all the stages of the growth.



FIG. 1. Symptoms on capitulum.

Repeated isolations from the infected plant parts yielded a dark olivaceous, richly sporulating fungus. The conidiophores are cylindrical often branched, 3 to 5 septate. Conidia are cylindrical to ellipsoid, pale brown, 1 to 12 septate, constricted at septa and measure  $32$  to  $112 \times 8$  to  $28 \mu$ . Longitudinal septa are rare. The fungus has been identified as *Alternaria helianthi* (Hansf.) Tubaki and Nishihara<sup>1</sup> by Dr. G. S. de Hoog. The specimen and culture have been deposited at MYSP herbarium and culture collection of University of Agricultural Sciences, Hebbal, with the accession Nos. MYSP 1781 and 90 respectively.

On inoculation of one-month old sunflower seedlings with conidial suspension, symptoms typical of disease were found 48 hours after the spray under humid conditions. Reisolations yielded the original fungus. This is a new record for India.

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#### EFFECT OF TIBA ON YIELD AND PHOTO-SYNTHETIC ENZYME IN RED GRAM

THE yield of soybeans and Bengal gram was enhanced by foliar spray of 2,3,5-triiodobenzoic acid (Green and Anderson, 1965; Sinha and Ghildiyal, 1973). No such effect was observed in *Lense esculenta* (Muchlbaner and Miller, 1971).

An experiment to examine the effect of TIBA (2,3,5-triiodobenzoic acid) on red gram (*Cajanus cajan*) var. BS 1 was conducted during the Kharif, 1971 under field conditions. The plot size was  $4\text{ m} \times 10\text{ m}$  and 10 liters of TIBA formulation Regim 8 supplied by the International Mineral and Chemical Corporation was sprayed at the time of first flower opening. In all there were five concentrations including a control as given in Table I.

TABLE I

Effect of 2,3,5-TIBA on the red gram var. BS-1

TIBA conc. mg/l	Yield kg/plot	% increase
0 Control	7.1	..
25	7.1	..
50	8.1	14
100	8.6	21
200	8.0	13

In addition, the effect of TIBA on RuDP carboxylase in leaves was also studied following the method of Bjorkman (1968).

The seed yield was enhanced by TIBA application (Table I). Amongst the five concentrations 0, 25, 50, 100 and 200  $\mu\text{g/ml}$  used, 100  $\mu\text{g/ml}$  gave the maximum yield increase of 21% over control.

When the leaves of the treated plants were assayed for RuDP carboxylase four days after spray, there was gradual decrease in the enzyme activity with the increase of TIBA concentration (Table II).

TABLE II

Effect of 2,3,5-triiodobenzoic acid on RuDP carboxylase activity in red gram

TIBA conc.	RuDP carboxylase activity $\text{CPM g}^{-1} \times \text{min}^{-1} \times 10^{-5}$
0 Control	37.69
25	31.74
50	22.97
100	22.93
200	12.52

The reaction mixture contained in  $\mu$  moles in total 0.5 ml: cysteine 1.25; EDTA 0.1, Tris HCl pH 8.1,  $10\text{ mg Cl}_2$  2.5, RuDP 0.15 and  $\text{NaHCO}_3$  2.5 containing  $0.5 \mu\text{C}^{14}$ .

Since this plant did not have any PEP carboxylase (Khanna *et al.*, 1971), it is unlikely that increase in yield was due to enhanced photosynthetic enzyme activity and consequently the photosynthesis rate. In soybeans the enhanced yield due to TIBA application is because of change in plant canopy structure which helps penetration of light to the lower leaves (Green and Anderson, 1965). This point needs verification in red gram. Alternatively, the application of TIBA might be responsible for better distribution of the dry matter produced by the plant before and after spray. The effect could also be on the nitrogen metabolism of plant which needs investigation.

Indian Agricultural  
Research Institute,  
New Delhi, December 11, 1973.

B. BALDEV.  
S. K. SINHA.

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**EFFECT OF SPRAYING SODIUM HUMATE AND HYDROQUINONE ON GLYCINE MAX VAR. BRAGG AND SOLANUM LYCOPERSICUM VAR. HEIZ, 1370**

HUMIC substances when incorporated in soil exert favourable influence on the growth and metabolism of plants resulting in appreciable crop yields<sup>1-4</sup>. The influence of spraying humic substances on legumes and vegetables in relation to nitrogen fixation and other related characteristics has not been reported. The reports on other crops have

also been scanty<sup>5,6</sup>. A microplot experiment started by the authors<sup>7</sup> in March, 1971 with *Phaseolus aureus* var. Pusa-Baisakhi showed that 10 ppm was the suitable concentration for spraying and that humic acid derived from farmyard manure was the best among the humic acids tested from different sources, viz., lignite, farmyard manure, wheat straw decomposed by *Trichoderma viride* or *Pullularia pullulans*. Humic acid from farmyard manure increased the grain yield by 77%. To confirm these findings in soybean (*Glycine max* var. Bragg) and tomato (*Solanum lycopersicum* var. Heiz, 1370) experiments were conducted in July, 1971 and October, 1971 respectively and the results are reported in this paper.

The experiment was conducted in microplot of 1 sq. meter in the sandy loam alluvial soil of Delhi (organic carbon 0.3%, total nitrogen 0.03% and pH 7.2). A basal dressing of superphosphate (80 kg P<sub>2</sub>O<sub>5</sub>/ha) was applied. Soybean seeds pelleted with peat based culture of *Rhizobium japonicum* strain SB 16 were sown. Sodium humate extracted from farmyard manure and hydroquinone in the form of aqueous solution (125 ml/plot) were sprayed at 10 and 50 ppm levels 4 times at a regular interval of 11 days keeping proper control. The moisture was maintained uniformly at 1/3 of the water holding capacity of the soil in all the plots. At maturity, grain yield was recorded and total nitrogen in grain and straw was determined by Kjeldahl's method.

Tomato crop was taken after soybean. Superphosphate (80 kg P<sub>2</sub>O<sub>5</sub>/ha) and ammonium sulphate (100 kg N/ha) were applied as basal dressing. The crop plants were sprayed 3 times with sodium humate and hydroquinone (125 ml/plot) at an interval of 20 days. The yield of tomato was recorded.

**TABLE I**

*Effect of spraying sodium humate and hydroquinone on grain yield and nitrogen uptake by Glycine max var. Bragg and on yield of Solanum lycopersicum var. Heiz, 1370*  
(Average of four replications)

Treatments	SOYBEAN				TOMATO
	Grain yield/plot (g)	Grain		Yield/Plot (g)	
		Nitrogen (%)	Nitrogen/ plot uptake (g)		
Control	.. 200.7	6.23	12.5	661.7	
10 ppm (Hydroquinone)	.. 218.5	6.15	13.4	1309.7	
50 ppm       "      "	.. 209.2	6.50	13.6	798.9	
10 ppm (Humate) "	.. 248.4	6.50	16.4	1383.4	
50 ppm (      "      ")	.. 229.0	6.60	14.6	1347.4	
C.D. at 5%	.. 6.17			157.9	



Ten parts per million sodium humate increased the grain yield of soybean by 24% whereas at 50 ppm, the increase was 14.5% only. Nitrogen uptake was also affected appreciably (Table I). The effect of spraying 10 ppm sodium humate was more marked on soybean var. Clark in a pot experiment<sup>8</sup>. In case of the tomato crop the yield was increased by 109 and 104% with the two levels of the humate (10 and 50 ppm) showing almost identical effects (Table I). Hydroquinone which was used as an alternative to the humate increased grain yield by 9 and 4.5% in soybean at 10 and 50 ppm respectively. The effect was more marked only in case of tomato sprayed with 10 ppm of hydroquinone where the yield was increased by 95%.

The physiological activity of humic substances may be explained both due to direct and indirect effects on plant growth. The beneficial action of humic compounds on plant is caused due to its functional group which take part in the metabolic processes of plant such as respiration, absorption

of nutrients and photosynthesis resulting in higher crop yields.

Thanks are due to Dr. N. S. Subba Rao for providing the necessary facilities.

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## INFORMATION TO CONTRIBUTORS

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## SHORT SCIENTIFIC NOTES

### A Note on Some Phytopathogenic Fungi from Hyderabad (India)

The author records the following phytopathogenic fungi occurring in Hyderabad District (A.P.): *Albugo ipomeae-panduratae* (Schwein) Swing on *Merremia emarginata* Hallier. f.; *Cercospora chloroxyli* Ramakrishnan and Reddy on *Chloroxylon swietenia* D.C.; *Macrophoma obsoleta* Sacc. on *Coccinia* sp.; *Pestalotiopsis japonica* (Syd.) Steyaert on *Terminalia tomentosa* W. and A.; *Phomopsis gardeniae* Buddin and Wakefield on *Gardenia lucida* Roxb.; *Puccinia lithospermi* E. and K. on *Evolvulus alsinoides* Linn. and *Stenella aegles* Prasad on *Aegle marmelos* Correa. Of these, *M. obsoleta*, *Phomopsis gardeniae* and *P. lithospermi* form new additions to the fungi of India. *Pestalotiopsis japonica* and *S. aegles* are new records for South India. *Albugo ipomeae-panduratae*, *Pestalotiopsis japonica* and *Cercospora chloroxyli* are new records for Andhra Pradesh State.

All the herbarium specimens of the above fungi are deposited at C.M.I., Kew, England and Mycology Laboratory, Botany Department, Osmania University, Hyderabad.

The author expresses his thanks to: the Director and Dr. Punithalingam, Mr. Deighton, Dr. Sivasenan and Dr. Mordue of C.M.I., Kew, England, for their help in identification, to Dr. P. Ramarao, Reader in Botany for guidance and to Prof. M. R. Suxena, Head, Department of Botany, Osmania University for encouragement.

Botany Department, C. MANOHARACHARY.  
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Hyderabad-500007, December 19, 1973.

### A TDC-12 Computer Program for the Computation of Bartlett's Test for Homogeneity of Variances

Bartlett's test has been given for testing the hypothesis  $H: \sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \dots = \sigma_k^2$ , where a random sample of " $n_i$ " observations had been taken

from the ' $i$ th' normal population ( $i = 1, \dots, k$ ) (Ostle, 1966)<sup>1</sup>. This test is statistically adequate for testing the homogeneity of variances. In the statistical analysis of field data, testing of homogeneity of variances is usually required by plant breeders in pooling error variances in groups of experiments. This is particularly true when they are interested in estimating and testing genotype  $\times$  environmental interactions. In estimating various stability parameters, this test is, however, of great use.

A computer program, intended for estimating Bartlett's test of homogeneity of variances, has been developed and documented for the TDC-12 computer.

The program is written in Fortran 4-k.

Detailed information regarding the use of the program and listing of the program can be obtained from us.

We are grateful to Dr. N. K. Anant Rao, Dean, Agriculture; Dr. K. G. Gollakota, Dean Post-Graduate Studies; and Dr. D. D. Pant, Dean, College of Basic Sciences and Humanities for facilities and encouragement.

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## REVIEWS AND NOTICES OF BOOKS

**Termite Problems in India.** Edited by Dr. M. L. Roonwal. (Published by Council of Scientific and Industrial Research, New Delhi), 1972. Pp. viii + 81. Price Rs. 9-00.

Termites are economically the most important and destructive pests which infest all materials in tropical countries. It is in fact easier to compile a list of materials which termites do not destroy than to enumerate the materials they destroy. The book under review highlights termite problems in India. The material of the book is based on the proceedings of a meeting of termitologists of India held on 26-27, March, 1970. 14 contributions by experts from various organisations are included in the book. The topics deal with the problem of termites in ordnance stores, forestry, Agriculture, Cash Crops, Horticulture, Sugarcane, etc. The scope and importance of cytogenetical work on termite systematics, casts differentiation, adaptation, and termite control, especially in Indian species, are indicated. Various aspects of termite physiology such as digestion, nutritional requirements, inhibitors for digestive enzymes, and utilisation of non-cellulosic materials and surplus glucose and the role of protozoan fauna in wood digestion by termites are some of the interesting and thought-provoking articles, a very useful material for future research workers. In the review on Laboratory Testing of Natural Termite Resistance of Indian Wood, salient features of development of acceleratory laboratory test in India have been brought out. Testing methods have been discussed. In the paper on Termite Control in Buildings, emphasis is laid on the protection measures during the stage of planning and construction. Apart from the use of soil treatment a search for effective and cheap digestive enzyme inhibitors is called for. Each article is

followed by a useful discussion on future lines of research. Recommendations adopted at the meeting deserve careful consideration from authorities that be.

It is an indispensable book and will be a valuable addition to the shelves of scientists, students and libraries and the authorities of the C.S.I.R. deserve all praise for organising such a useful meeting and bringing out the proceedings. Unfortunately, such useful information became available almost after two years of the meeting which should have been avoided.

V. R. SIVARAMAKRISHNAN.

### Books Received

- Fixation in Histochemistry.* Edited by P. J. Stoward. (Chapman & Hall Ltd., London EC4P 4EE), 1973. Pp. xiii + 201. Price £5.00.
- Progress of Plant Ecology in India* (Vol. I). Edited by R. Misra, B. Gopal, K. P. Singh and J. S. Singh. (To-day and Tomorrow's Printers & Pub., 23-B/5, Original Road, Karol Bagh, New Delhi 110005), 1973. Pp. 153. Price Rs. 40/- or \$7.00.
- Ornamental Horticulture in India.* By G. S. Randhawa. (To-day and Tomorrow's Printers & Pub., 22 B/5, Original Road, New Delhi 110005), 1973. Pp. 142 + 13. Price Rs. 40/- or \$8.00.
- Imbedding Methods in Applied Mathematics.* By John Casti and Robert Kalaba. (Addison-Wesley Pub. Co., Reading, Massachusetts 01867, USA), 1973. Pp. xiv + 306. Price : \$16.00, \$8.50.
- Chemical Thermodynamics—Basic Theory and Methods* (3rd Edn.), By Irving M. Klotz and R. M. Rosenberg, (W. A. Benjamin, Inc., Menlo Park, California, Addison-Wesley Pub. Co., Reading Mass. 01867), 1972. Pp. xvi + 444. Price \$15.60.

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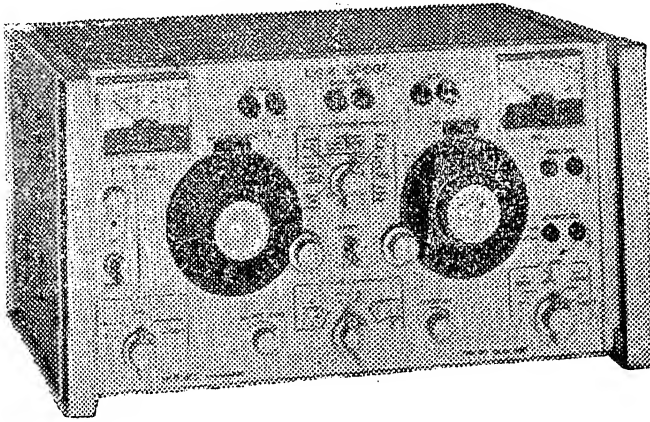
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# ON THE SHAPE AND STABILITY OF FINGERS IN A DISPLACEMENT PROCESS THROUGH A FRACTURED POROUS MEDIUM

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## ABSTRACT

A specific problem of fingering occurring in an immiscible displacement process through a slightly fractured porous medium is discussed here from a statistical view point. A mathematical solution has been obtained by using a perturbation technique and an expression for the average cross sectional area occupied by the fingers is obtained. It is shown that stabilization of fingers may occur in this particular case.

## INTRODUCTION

IT is often important for various Engineering and Hydro-geological problems to know whether the displacing fluid forms a stable front during the displacement process or whether it will spread rapidly through the displaced fluid forming an unstable front. Instead of the displacement of the front as a whole in regular form, protuberances occur that may advance through the porous medium at velocities much higher than the average front. This instability phenomenon is known as 'fingering' and occurs when a fluid of lesser viscosity displaces another of higher viscosity.

The growth and stability of fingers in homogeneous porous media was analysed from statistical view point by Scheidegger and Johnson<sup>1</sup>, and they found that no stabilization of the fingers is possible in the statistical theory. Subsequently many authors for instance Chouke *et al.*<sup>2</sup>, Marle<sup>3</sup>, Verma<sup>4,5</sup>, Venkateswarlu<sup>6-8</sup> have investigated the phenomenon of fingering and specific double phase flow problems in porous, and fractured media. The basic assumption underlying the present investigation is that the porous medium is slightly fractured and the fractures are randomly oriented. To make the analysis more definite we consider that the finger flow is furnished by water displacing oil from a one-dimensional fractured medium. By using a perturbation procedure, it is shown that the stabilization of fingers may occur in this particular case.

## STATEMENT OF THE PROBLEM

Consider a homogeneous porous medium saturated with oil and containing randomly oriented fractures. Water at a constant velocity ( $V$ ) is injected into the fractured medium. The displacement of oil by water gives rise to a well developed system of finger flow (Fig. 1). It is assumed that due to the impact of the injecting water the entire oil on the initial boundary  $x = 0$  ( $x$  being measured in the direction of displacement) is displaced through a small distance. Thus at  $x = 0$ ,  $K_0 = 0$  is the boundary condition of the problem.

## STATISTICS OF FINGERS

In the statistical approach only the average cross sectional area occupied by the fingers is taken into consideration while the size and shape of the individual fingers is discarded. This treatment with the introduction of notion of fictitious relative permeability becomes identical to the Buckley and Leverett description of the immiscible double phase flow (Scheidegger *et al.*<sup>1</sup>). In this case, the saturation of the  $i$ -th fluid ( $S_i$ ) is defined as the average cross sectional area occupied by it at the level  $x$ , i.e.,  $S_i = S_i(x, t)$ . As such the saturation of the displacing fluid in the porous medium represents the average cross sectional area occupied by the fingers.

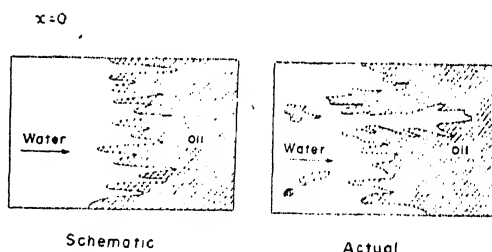


FIG. 1. Fingering in an immiscible displacement process.

Following Scheidegger and Johnson<sup>1</sup>, we consider the fictitious relative permeability to water ( $K_w$ ) and oil ( $K_o$ ) as :

$$K_w = S_w, \quad K_o = S_o \quad (1)$$

where  $S_w$  and  $S_o$  denote the saturation of water and oil.

In a fractured porous medium the water entering the fractures is sucked into the blocks under the capillary action. The amount of water entering the blocks in an elementary volume of the medium is known as the 'Impregnation function'  $\phi(t)$ . Considering the balance of water sucked into the blocks per unit time and employing the results of

Mattax and Kyte<sup>9</sup>, we may write the analytical value of the impregnation function  $\phi(t)$  as below:

$$\phi [T - \tau(u)] = D (T - Rx^2)^{-1/2}; \tau \leq t \quad (2)$$

$$u = \frac{x}{l}; \quad T = \epsilon t; \quad R = \frac{a}{l^2}$$

$$\epsilon = \frac{\sigma \cos \theta S^2 \sqrt{K/m_B}}{\mu_0}$$

$$a = \left( \frac{\pi A S^2 g_K \sigma \cos \theta m_B \sqrt{K/m_B}}{4Q \mu_0} \right)^2$$

The symbols are defined at the end of article.

#### FORMULATION OF THE PROBLEM

Assuming the flow to be governed by Darcy's law, the seepage velocities for water ( $v_w$ ) and oil ( $v_o$ ) and the continuity equations for the flowing phases can be written as:

$$v_w = -K \frac{K_w}{\mu_w} \frac{\partial p}{\partial x} \quad (3)$$

$$v_o = -K \frac{K_o}{\mu_o} \frac{\partial p}{\partial x} \quad (4)$$

$$m \frac{\partial S_w}{\partial t} + \frac{\partial v_w}{\partial x} + \phi [T - \tau(u)] = 0 \quad (5)$$

$$m \frac{\partial S_o}{\partial t} + \frac{\partial v_o}{\partial x} - \phi [T - \tau(u)] = 0 \quad (6)$$

where  $m$  and  $k$  are the porosity and permeability of the medium.

From the definition of phase saturation, it is evident that:

$$S_w + S_o = 1. \quad (7)$$

Combining equations 5 and 6 and using 3, 4 and 7, we have:

$$\frac{\partial}{\partial x} \left[ K \frac{\partial p}{\partial x} \left\{ \frac{K_w}{\mu_w} + \frac{K_o}{\mu_o} \right\} \right] = 0 \quad (8)$$

Integrating the above equation with respect to  $x$  under the boundary condition of our problem, viz.,

$$K_o(0, t) = S_o(0, t) = 0;$$

$$- \left( K \frac{K_w}{\mu_w} \frac{\partial p}{\partial x} \right)_{0,t} = v_w(0, t) = V$$

we get.

$$\frac{\partial p}{\partial x} = - \frac{V}{K \left[ \frac{K_w}{\mu_w} + \frac{K_o}{\mu_o} \right]} \quad (9)$$

The values of  $\partial p / \partial x$  and  $v_w$  may be used in equation 5 and write the equation of motion for the displacing phase saturation as follows:

$$m \frac{\partial S_w}{\partial t} + V \frac{\partial}{\partial x} \left[ \frac{\frac{K_w}{\mu_w}}{\frac{K_w}{\mu_w} + \frac{K_o}{\mu_o}} \right] + \phi [T - \tau(u)] = 0. \quad (10)$$

Since  $K_w$  and  $K_o$  are the functions of  $S_w$ , we rewrite the equation of motion with the help of equation 1, as under:

$$m \frac{\partial S_w}{\partial t} + V \left[ \frac{P}{(1 - S_w + PS_w)^2} \right] \frac{\partial S_w}{\partial x} + \phi [T - \tau(u)] = 0 \quad (11)$$

where

$$P = \mu_o / \mu_w$$

#### SOLUTION BY PERTURBATION METHOD

As the medium is slightly fractured, we assume the capillary suction function  $\phi [T - \tau(u)]$  that was included in the continuity equations due to the presence of fractures is a small quantity. Therefore we use it as a perturbation parameter to solve the equation of motion for saturation. Thus neglecting  $\phi [T - \tau(u)]$  in equation 11, we have:

$$m \frac{\partial S_w}{\partial t} + V \left[ \frac{P}{(1 - S_w + PS_w)^2} \right] \frac{\partial S_w}{\partial x} = 0. \quad (12)$$

The characteristic equations of equation 12 are:

$$\frac{dS_w}{dx} = 0$$

and

$$\frac{dx}{dt} = \frac{V}{m} \left[ \frac{P}{(1 - S_w + PS_w)^2} \right] \quad (13)$$

Integrating the above characteristics equations under the boundary condition that  $x = 0, t = 0$ , we have:

$$x = \frac{Vt}{m} \left[ \frac{P}{(1 - S_w + PS_w)^2} \right]. \quad (14)$$

Changing  $t$  to  $T$  and substituting the value of  $\phi [T - \tau(u)]$  from equation 2, we may write equation 11 as follows:

$$m\epsilon \frac{\partial S_w}{\partial T} + V \left[ \frac{P}{(1 - S_w + PS_w)^2} \right] \frac{\partial S_w}{\partial x} + \frac{D}{\sqrt{T - Rx^2}} = 0. \quad (15)$$

Evgenyev<sup>10</sup> has pointed from his experimental observations that in most cases  $P$ , the ratio of viscosity of oil to water is large and therefore we may regard  $1/P$  as a small quantity. We substitute the value of  $T (= \epsilon t)$  as obtained from equation 14 in the last term of equation 15 and after simplifying in view of the above remark, we obtain:

$$m\epsilon \frac{\partial S_w}{\partial T} + V \left[ \frac{P}{(1 - S_w + PS_w)^2} \right] \frac{\partial S_w}{\partial x} + D \left( \frac{VP}{m\epsilon} \right)^{1/2} \frac{\bar{x}^{1/2}}{(1 - S_w + PS_w)} = 0. \quad (16)$$

This is a quasi-linear equation of motion whose characteristic equations are :

$$\frac{dx}{dt} = \frac{V}{m} \left[ \frac{P}{(1 - S_w + PS_w)^2} \right],$$

$$\frac{dS_w}{dx} = \frac{D \left( \frac{VP}{m\epsilon} \right)^{\frac{1}{2}} (1 - S_w + PS_w)^{\frac{1}{2}}}{V \left[ \frac{P}{(1 - S_w + PS_w)^2} \right]}. \quad (17)$$

The equivalent form of the above equations can be written as follows :

$$D \left( \frac{VP}{m\epsilon} \right)^{-\frac{1}{2}} x^{-\frac{1}{2}} dx = \frac{VP}{(1 - S_w + PS_w)} dS_w \quad (18)$$

$$\frac{dt}{m} = \frac{1}{D} \left( \frac{m\epsilon}{VP} \right)^{\frac{1}{2}} x^{\frac{1}{2}} (1 - S_w + PS_w) dS_w. \quad (19)$$

Integration of equation 18 gives :

$$2D \left( \frac{VP}{m\epsilon} \right)^{\frac{1}{2}} x^{\frac{1}{2}} \\ = \frac{VP}{(P - 1)} \log (1 - S_w + PS_w) + E \quad (20)$$

where E is a constant of integration.

Similarly integrating equation 19 with the help of equation 20 we get :

$$t = \frac{m\epsilon^2}{4D^2(P-1)} [(1 - S_w + PS_w)^2 \\ \times \log (1 - S_w + PS_w)] \\ - \frac{m^2\epsilon}{8D^2(P-1)} (1 - S_w + PS_w)^2 \\ + E \frac{m^2\epsilon}{4D^2VP} \cdot S_w [1 + (1 - S_w + PS_w)] \\ + F \quad (21)$$

where F is a constant of integration. An arbitrary functional relation between these two integrals gives the solution of equation 16.

Since the saturation  $S_w$  is defined as the average cross-sectional area occupied by the fingers, we may consider  $S_w = 0$  as the criterion for investigating the stability of the fingers. Thus putting  $S_w = 0$  in the equations 20 and 21, we note that definite values of  $x$  and  $t$  correspond to zero value of  $S_w$ , and this in turn implies that the stabilization of fingers is possible in the specific problem investigated.

It may be remarked here that the conclusions drawn depend on the perturbation procedure which has been used here. Notwithstanding the difficulty in using such a procedure for studying the long term behaviour of the solution (Scheidegger<sup>11</sup>), we have adopted it due to the special nature of the medium and the particular qualitative interest of the present investigation, viz., showing the occurrence of stable

fingers in one case of queer permeability-homogeneity and capillary suction term.

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List of Symbols used :

A = a constant

$g_k$  = saturation of blocks with water at the moment  $t_k$

$k$  = permeability of the fractured system

$k_w$  = relative permeability to water

$k_o$  = relative permeability to oil

$l$  = mean block size

$\rho$  = viscosity ratio

$m_w$  = porosity of blocks

$m$  = porosity of the fractured system

$q$  = average rate of flow across the striking face

$S$  = mean specific surface area of the blocks

$S_w$  = saturation of water

$S_o$  = saturation of oil

$t$  = time

$T$  = weighted time

$u$  = mean coordinate

$v_w$  = seepage velocity of water

$v_o$  = seepage velocity of oil

$x$  = linear coordinate

$\mu_w$  = viscosity of water

$\mu_o$  = viscosity of oil

$\sigma$  = surface tension

$\epsilon$  = a complex constant of fractured characteristics and oil viscosity

$\phi(t)$  = impregnation function

$\theta$  = wetting angle.

Some constants occurring in the definition of the impregnation function, viz.,  $a$ ,  $D$  and  $R$  are not shown in this table.

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## SOME RESULTS OF GEOPHYSICAL SURVEYS IN PONDICHERRY AND ADJOINING AREAS OF SOUTH ARCOT DISTRICT, TAMIL NADU

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### ABSTRACT

The present paper deals mostly with the results of the seismic refraction surveys conducted during the Field Season 1969-70 in Pondicherry and adjoining parts of South Arcot District, Tamil Nadu for groundwater. About 122 electrical resistivity probes employing Schlumberger (Maximum AB/2-250 m) and 22 long range refraction seismic profiles (Maximum spread of 1809 m) were spaced evenly in an area of 350 Sq. Kms. The surveys have brought out valuable information on the nature of the sediments and the general disposition of the crystalline basement in and around Pondicherry.

### INTRODUCTION

IN view of the drought conditions in Pondicherry State during 1969, geophysical surveys comprising seismic refraction and electrical resistivity soundings were carried out with a view to assist the tube-well drilling programme in this area. The electrical surveys were not successful in delineating the relatively more favourable granular zones within the Tertiary sandstones, cropping out around Pondicherry since they are overlain by a thick cover of laterite which is highly resistive and at times posed a severe problem of imparting current into the ground. These surveys have also failed in places where there is a thick cover of clay or very fine grained sands. However, the resistivity surveys have been useful chiefly in picking up the basement wherever shallow. Long range refraction seismic profiling was carried out to get the information on the thickness of the sediments and their nature in the deeper portions of the basin. The following account relates mostly to the results of seismic surveys

as these have been chiefly useful not only in delineating the basement topography in and around Pondicherry but also in indicating the nature of the overlying sediments for the purpose of groundwater exploration. This information on the total sedimentary column in this area should be useful contribution to the development of groundwater.

Geologically, Pondicherry is located in the well-known coastal sedimentary belt of Tamil Nadu, occupied chiefly by the Cretaceous and Tertiary rocks (Fig. 1). In view of the occurrence of tertiary coal (Lignite) around Neyveli and the possible oil bearing potential of the area, geophysical surveys were initiated in this belt nearly two decades ago (Kailasam, 1954) by the Geological Survey of India. The Oil and Natural Gas Commission has also been active in this belt for the past few years. It may be mentioned incidentally that the recent drilling results of the Geological Survey of India and Oil and Natural Gas Commission around Pondicherry were of help in the correlation of geophysical results.

### GEOLOGICAL FEATURES

The geological succession of Cretaceous and Tertiary rocks along the east coast is as follows: (Krishnan, M.S., 1942).

Quaternary	Recent	.....	Laterite, Coastal Sands Clay.
Tertiary	Miocene	.....	Sandstones, Clays, Sands, etc.
Cretaceous	Niniyur	.....	Sandy limestones, Sands, Calcareous nodules.
	Ariyalur	.....	Shell limestone, Calcareous Sandstones, and Shale.
	Trichinopoly	.....	Shell limestone, Silicified tree trunks, Sandstones, Clays, Conglomerates, Grits.
Archaeans	Charnockites		

Among the formations exposed in the area, the Niniyurs consist of argillaceous sandstones and sandy limestones. The Ariyalurs exposed at Valuvadur are represented by highly fossiliferous

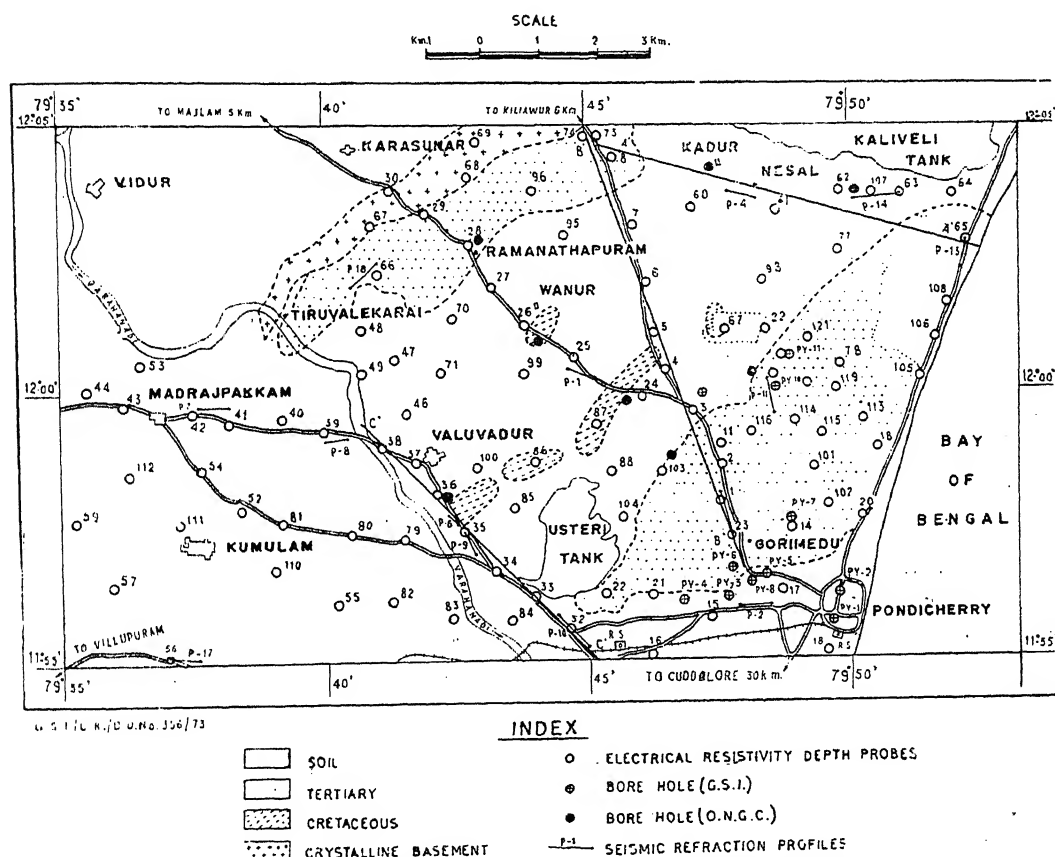


FIG. 1. The Layout of the geophysical survey together with the geology of Pondicherry and adjoining areas of South Arcot District, Tamil Nadu.

Near Pondicherry, the Cuddalores rest on the Niniyur or Ariyalur formations while further to the west they lie directly on the Trichinopoly beds of marine origin. The Cuddalores also rest directly on the crystallines at places overlapping the Cretaceous rocks. These sediments have gentle south-eastern dips of 2 to 10.

calcareous sandstones and shales. The Trichinopoly rocks, which are in general arenaceous, are exposed near Tiruvakarai which is noted for its silicified tree trunks. Cuddalores are mostly ferruginous sandstones that are sometimes argillaceous. According to Pascoe, 'The Cuddalores are derived from the silts by many rivers, which debouch into

the Bay of Bengal, mixed with wind blown material, and distributed along the coast by lateral currents'.

#### SUMMARY OF RESULTS

Seismic profiles shot over exposures of the various geological formations have yielded the following formational velocities:

Formation	Longitudinal Velocity
Alluvium or soil	.. 400 to 800 m/sec
Cuddalore Sandstones	.. 1,600 to 2,200 m/sec
Niniyur Sandstones	.. 2,100 to 2,500 m/sec
Ariyalur (Calcareous Sandstones)	2,800 to 3,100 m/sec
Crystalline Basement	.. 5,000 to 6,000 m/sec

As is to be expected from the above velocities, there has been no difficulty in recognising the

#### Case I-Near Kolavari

#### ONGC-BOREHOLE LOG

Limestone  
----- 30 m

Claystone with bands of fine to medium grained sands.  
----- 200 m

crystalline basement with respect to the overlying sediments. Similarly it was possible to differentiate between the top few metres of alluvium and the underlying Cuddalore sandstones. However, in view of the over-lapping of velocities of Cuddalores (Tertiary) and Niniyur (Cretaceous) sandstones, there has been some measure of uncertainty in recognising the Tertiary-Cretaceous interface at depth from the seismic results. For instance, while the bore-holes near Aurocentre (G.S.I. Py-10) and Valuvadur (O.N.G.C.) have clearly passed through the Tertiary and Cretaceous formations, their interface has not been indicated in the seismic refraction profiles shot in the vicinity. It was however possible to differentiate between these strata generally along section BB where corroboration was also available from a Geological Survey of India bore-hole Py-6. Again the Niniyur-Ariyalur interface within the Cretaceous sequence could be recognised invariably as these two formations differ appreciably in their velocities.

A typical time-distance curve which has picked up different velocities corresponding to the above mentioned formations is illustrated in Fig. 2.

The depth sections pertaining to three of the longer seismic profiles are presented (Fig. 3). Section AA and CC indicate a rather sloping down on the basement towards the coast. The maximum

depth to the basement in the case of section AA is of the order of 350 m. Beyond profile P-9 on section CC, the basement in fact could not be picked up even with a maximum shotdetector spread of 1.8 km. Section BB is however different from the other two indicating as it does only a small variation of 280 to 320 m in the basement depths. Another interesting feature may be recognised in sections AA and BB. The basement appears to drop very steeply for the first few kilometres from where the Archaeans are exposed. Hence along these profiles the contact of the Archaeans with the sedimentaries further to the east could possibly be a faulted one.

It may be of interest to study some of the seismic results with the available bore-hole data:

#### SEISMIC DATA

1,000 m/sec (Weathered zone)  
----- 8 m

3,400 m/sec limestone  
----- 350 m

6,400 m/sec Crystalline basement.

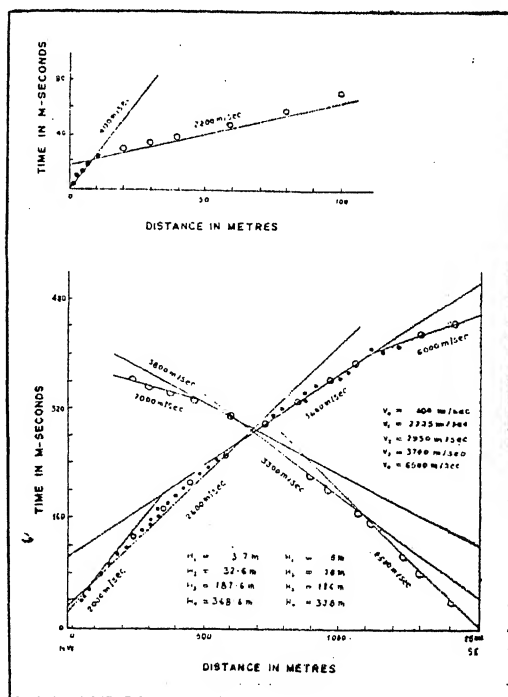
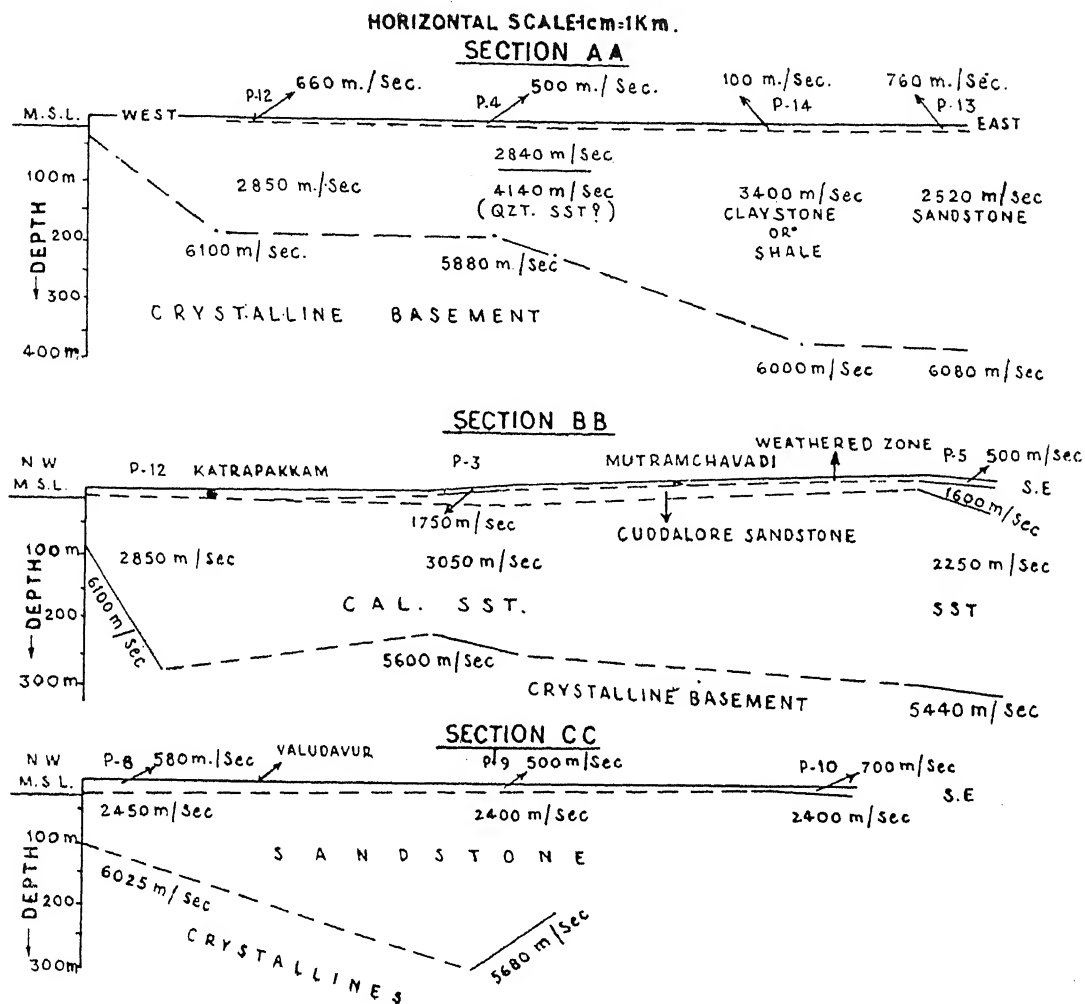


FIG. 2. A typical Time-distance curve over Profile-1 Near Sedarpeth, Pondicherry State.



G.S.I./C.R./D.O.No.357/73

FIG. 3. Seismic refraction depth sections AA, BB and CC in Pondicherry and adjoining areas of South Arcot District, Tamil Nadu.

Before comparing the logs it may be borne in mind that the borehole and the point where the seismic depths were estimated do not generally coincide. That is why a weathered zone of 8 m thick was picked up in the seismic profile while it was not encountered in the borehole. The limestone bed in the borehole may be correlated with the velocity of 3400 m/sec. It may be marked here that the layers below the limestones in the borehole have not been picked up in the seismic profiles as they form low velocity layers. These layers may result in the increase of the estimated depths.

The Calcareous sandstones indicated in the borehole upto a depth of 24 m may be correlated to longitudinal velocity of 2840 m/sec at a depth of 4 m.

The intermediate velocity of 4140 m/sec may possibly correspond to quartzitic sandstone and its depth calculated (80 m) is more than the actual depth (60 m). This perhaps resulted due to the presence of low velocity layers such as clays and fine grained sands which are clearly indicated in the borehole.

From the hydrological point of view, the formations indicated along sections BB and CC are

## Case II

## ONGC-BOREHOLE (KADUR)

Calcareous Sandstones	24 m
Claystone	36 m
Sand fine to medium	60 m
Quartzitic sandstone	129
Claystone, alternate bands of fine to medium grained sands.	200 m.

## SEISMIC DATA

(near Nesal, falling close to Kadur)

540 m/sec	4 m
2,840 m/sec (Calcareous sandstone)	80 m
4,140 m/sec	
Quartzitic sandstone	210 m
5,880 m/sec (Crystalline)	

likely to be more promising generally. They should consist largely of sandstones, whereas along section AA only limestones and shales may predominate. This inference is based upon the longitudinal velocities obtained and the available borehole information.

On the basis of the seismic and to some extent the resistivity results, the subsurface contour map of the crystalline basement reduced to the mean sea level has been prepared (Fig. 4). This clearly indicates a rapid sloping down of the basement

towards the coast. The sediments are likely to attain their highest thickness in the southeastern corner of the area which is to be expected from geological considerations also. This thickness as estimated from seismic results should be of the order of 350 m.

## CONCLUSIONS

The seismic surveys have clearly brought out the general configuration of the basement and the nature of the overlying sediments.

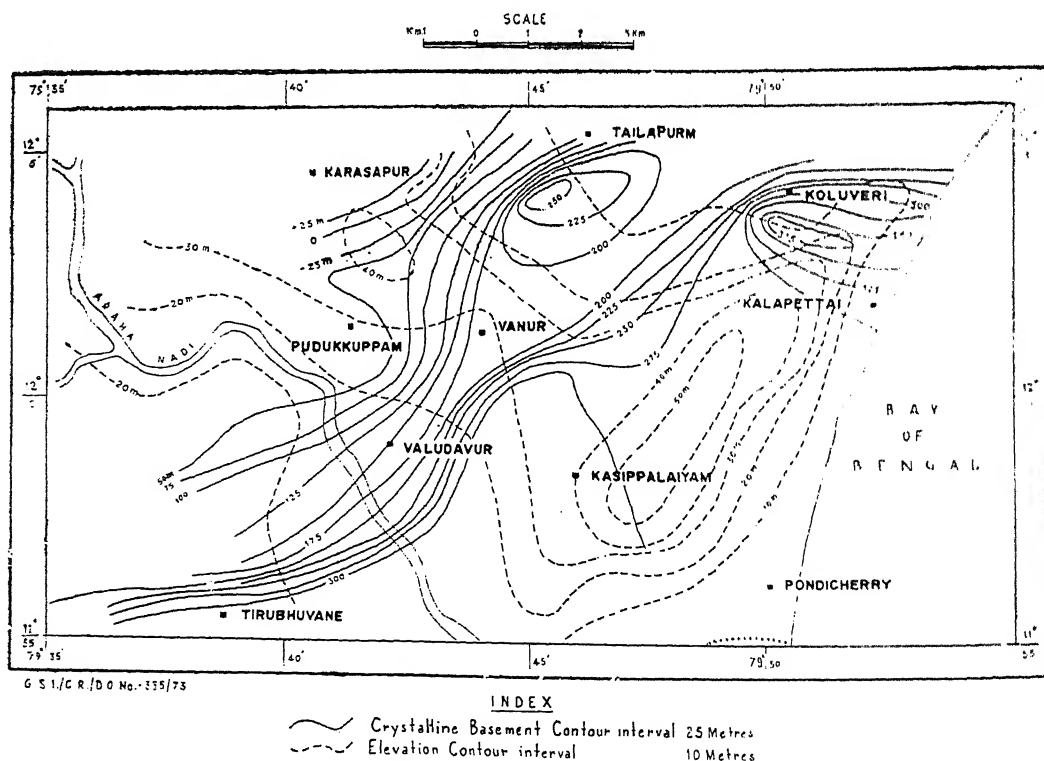


FIG. 4. Configuration of the crystalline basement inferred from the geophysical surveys in Pondicherry and adjoining areas of South Arcot District, Tamil Nadu.

Seismic depth estimates arrived at do not take into consideration the possible presence of low velocity layers which are not unlikely in the area as revealed by some of the boreholes.

#### ACKNOWLEDGEMENTS

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## DEFORMATION BEHAVIOR OF POLYCRYSTALLINE ZINC AT 4.2° K

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A STUDY of the deformation characteristics of metal at very low temperatures is of considerable interest in view of the restricted occurrence of thermally activated slip processes. This is particularly so in hexagonal close packed (h.c.p.) metals because of the existence of only a limited number of operative slip systems. Several h.c.p. metals such as Be, Zn and Mg are brittle at low temperatures<sup>1</sup> and therefore their deformation behavior upto a reasonable magnitude of strain can be studied only under compression. In this study the nature of plastic deformation of polycrystalline zinc including strain rate effects under compression at 4.2° K is reported.

Cylindrical specimens with 5 mm diameter and 13.6 mm height were machined from hot rolled zinc material (99.98%) and were annealed at 150°C for one hour in a paraffin oil bath. The resulting average grain diameter was 0.036 mm. Compression testing was carried out on an Instron machine employing a compression jig which uses spherical ball bearings for the axial application of load to the specimen. The tests at 4.2° K and 77° K were conducted by surrounding the specimen

with liquid helium and nitrogen, respectively, contained in suitable cryostats.

The force versus cross-head displacement curves recorded for the zinc material at 4.2° K and 77° K are shown in Fig. 1. After the material was

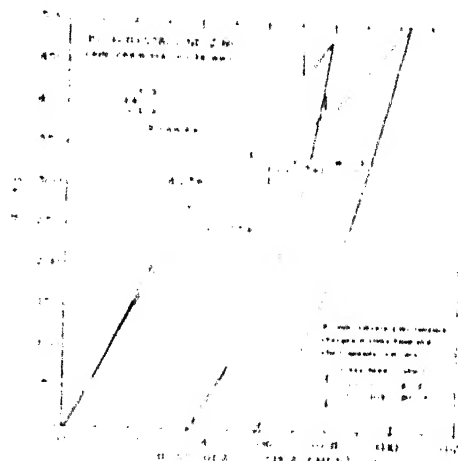


Fig. 1. Load versus cross-head displacement curves recorded on polycrystalline zinc material deformed at 4.2° K and 77° K under compression.

deformed to some plastic strain, the cross-head speed and chart speed were changed by one order of magnitude with a view to examining the effect

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of a strain rate change. The load—displacement curves show the following striking features:

- (i) The apparent work hardening rate of the material is very large, being larger at 4.2° K ( $\approx 340 \text{ kg/mm}^2$ ) than at 77° K ( $\approx 240 \text{ kg/mm}^2$ ), and is about one-tenth of the shear modulus of zinc material.
- (ii) The strain rate sensitivity of the flow stress at 4.2° K is nearly zero, in spite of the high work hardening rate. This implies that the activation volume,  $v^*$ , given by:

$$v^* = m (\Delta \ln \dot{\epsilon} / \Delta \sigma)_T RT, \quad (1)$$

which is a parameter commonly used to express the effect of strain rate ( $\dot{\epsilon}$ ) on the flow stress ( $\sigma$ ) at constant temperature ( $T$ ), is therefore nearly zero at 4.2° K. In equation (1),  $R$  is the gas constant and  $m$  is an orientation factor approximately equal to 6.5. The activation volume from measurements at 77° K, was calculated to be,  $3 \times 10^{-18} \text{ mm}^3$ .

- (iii) The stress at which permanent deformation occurs at 4.2° K is nearly the same as at 77° K.

These features clearly indicate that the deformation process occurring at 4.2° K is essentially athermal in nature and hence the occurrence of mechanisms based on thermally-activated slip processes can be ruled out. Alternately, deformation twinning and/or cracking are to be considered. At these low temperatures, mechanical twinning occurs in competition to slip since the resolved shear stress for slip is large under these conditions. Moreover, because of the restricted number of slip systems, mechanical twinning is an important mode of deformation in h.c.p. metals at low temperatures. It is known, in fact, that  $\{10\bar{1}2\} \{10\bar{1}1\}$  twins occur in zinc even at relatively higher temperatures<sup>2</sup>. As deformation twinning is a shear process involving cooperative movement of atoms through a material volume within microseconds, the temperature and strain rate do not appear to have an

appreciable influence on the flow stress and hence the deformation caused by twinning can be considered as an essentially athermal process. This appears to be a good possibility of accounting for the apparent zero activation volume observed in the present investigation. In addition to the twinning process, it is also possible that the yielding of this material under compression is associated with the nucleation and growth of cracks which are again normally taken to be athermal processes. The propagation of cracks is thought to be rather slow under compression as a result of which the material exhibits reasonable ductility before fracture. Thus twinning as well as cracking processes might be responsible for the apparent athermal nature of deformation of this material at 4.2° K.

It is perhaps interesting to compare the deformation behavior of zinc material with that of another h.c.p. metal deformed at 4.2° K, namely, titanium. Madhava and Armstrong<sup>3</sup> have observed that polycrystalline titanium exhibits discontinuous deformation at 4.2° K in a somewhat similar manner to that found by Madhava, Worthington and Armstrong<sup>4</sup>, in steel material. Deformation twinning was suggested by these workers as the probable mechanism for this observation. In zinc material, however, the occurrence of cracking is an added consideration besides deformation twinning.

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# KINETICS OF OXIDATION OF *p*-NITROBENZALDEHYDE BY POTASSIUM PERMANGANATE

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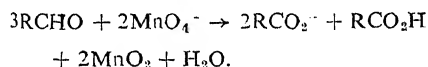
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ALTHOUGH  $\text{KMnO}_4$  is one of the most important oxidising agents in laboratory practice, the kinetics of oxidation of Aromatic aldehyde by neutral potassium permanganate have received little study. Tronov<sup>1</sup> determined the rate of reaction between  $\text{KMnO}_4$  and a number of aldehydes under limited conditions, but he did not attempt to interpret his data in terms of a mechanism. Tompkins<sup>2</sup> found that the oxidation of benzaldehyde with  $\text{KMnO}_4$  showed a linear increase of rate with increasing hydroxyl ion concentration. Wiberg<sup>3</sup> studied a number of aromatic aldehydes with neutral and alkaline medium and proposed a mechanism. A recent communication on the oxidation of *p*-nitrobenzaldehyde by  $\text{KMnO}_4$  in acetic acid medium,<sup>4</sup> prompts us to record our work on the oxidation of *p*-nitrobenzaldehyde in different buffers in neutral medium and at different temperatures.

All the reagents used were AnalaR grade and *p*-nitrobenzaldehyde was of Fluka make. The reaction mixture consisted of 38 ml of aldehyde (Ca  $2.5 \times 10^{-3}$  M), 10 ml of different buffers and 2 ml of  $\text{KMnO}_4$  (Ca  $1 \times 10^{-2}$  M). The buffer used were phosphate, pH range 5.5–11.0 and concentrations varied between .009 M to .06 M. Acetate and bicarbonate buffer for pH 6.5 and concentration was between .009 M to .06 M. The pH of the solution was adjusted to the required value by the addition of small amounts of diluted KOH or diluted  $\text{H}_2\text{SO}_4$  before the addition of  $\text{KMnO}_4$ . Aliquot quantity of the reaction mixture was pipetted out at successive time intervals into a known excess of acidified KI and liberated iodine was titrated with standard sodium thiosulfate. The over all stoichiometry of the reaction is



The reaction was found to be first order in permanganate, first order in aldehyde, and the over-

all second order constant was calculated by the equation :

$$k = \frac{2.303}{t(a-b)} \log \frac{b(a - 3/2 x)}{a(b - x)}$$

where 'a' and 'b' are the initial concentration of aldehyde and permanganate respectively. The experiments were done at different pH using a particular buffer and also at a constant pH using various concentrations of buffers. The temperature range was between 11–40° C.

It was observed that the oxidation of aldehyde is pH dependent as shown in Fig. 1. At lower pH

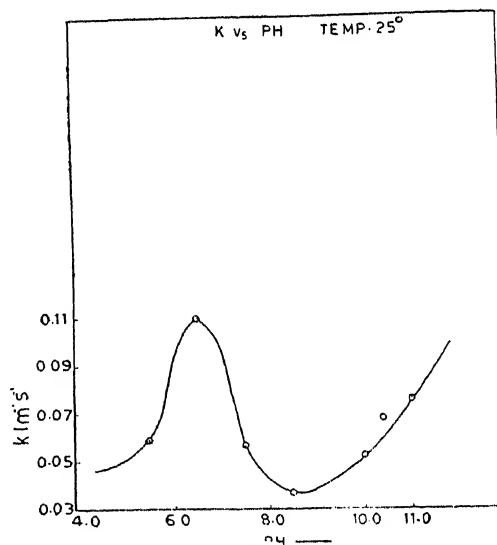


FIG. 1

there is autocatalytic reaction and in the alkaline region (pH 10–12) the rate is directly proportional to the square root of hydroxyl ion concentration. The curve indicates different types of mechanism are operative in the different pH region. The rate constants and other thermodynamic parameters as a function of pH is given in Table I.

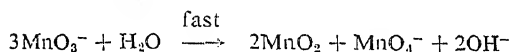
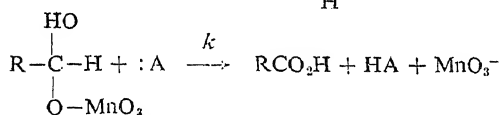
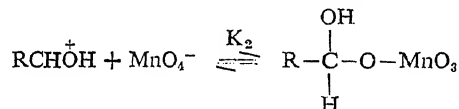


TABLE I

Thermodynamic parameters at 25° C in .02 M  $\text{NaH}_2\text{PO}_4$  buffer at different pH  $\mu = .004$

pH	$K_{25^\circ\text{C}} \text{ M}^{-1} \text{ Sec}^{-1}$	$\Delta E$ Kcal/mole <sup>1</sup>	$\Delta S$ Cal/mole
8.5	.03770	9.15	-34.3
7.5	.05757	8.47	-35.4
6.5	.1109	7.32	-38.4
5.5	.05970	7.04	-40.5

Since it was suggested<sup>3</sup> that this reaction may also undergo general acid catalysis, experiments were done at different concentration of buffers and at a constant pH 6.5. Wiberg has proposed the following mechanism for oxidation in the neutral medium :



If  $k_1$ ,  $k_2$  and  $k_3$  are catalytic constants for acidic, neutral and general acid catalysis, then it can be shown the over all rate :

$$V = K_1 K_2 [\text{RCHO}] [\text{MnO}_4^-] \{k_1 [\text{H}_3\text{O}^+] + k_2 K_{10} + k_3 K_i [\text{HA}]\}$$

or

$$V = K_{11} [\text{RCHO}] [\text{MnO}_4^-] \\ = k_{11} = K_1 K_2 \{k_1 [\text{H}_3\text{O}^+] + k_2 K_{10} + K_i k_3 [\text{HA}]\}$$

so that at a constant pH and at constant temperature, the plot of second order rate constant *versus* the molar concentration of general acid should be linear and the slope will give  $K_1 K_2 k_3 K_i$  where  $K_i$  is the ionisation constant of the acid. The slope will be different for different acids. This has been verified for phosphate, acetate and bicarbonate buffers. From the slope it is not possible to calculate  $k_3$ , the catalytic constant for general acid catalysis, separately. However, knowing  $K_i$  ionisation constant of the general acid, the relative values of  $k_3$  in different buffers can be obtained. The values so calculated are :

$$k_{\text{NaH}_2\text{PO}_4} : k_{\text{KHCO}_3} : k_{\text{NaAc}} :: 1 : 0.32 : 0.007$$

indicating that the phosphate catalysis the reaction much faster than bicarbonate or acetate.

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## INFORMATION TO CONTRIBUTORS

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Manuscripts, to be submitted in duplicate, should be typewritten in double space on one side of the paper, carefully revised by the authors and in final form for printing. Illustrations should be minimum in number, drawn in black and white with Indian ink on bristol board, and preferably enlarged to about twice the size they are intended to appear in print which will be mostly in column size.

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## LETTERS TO THE EDITOR

### CRYSTAL DATA OF MONONUCLEOTIDES— DEOXYGUANOSINE-5' PHOSPHATE AND GUANOSINE-5' PHOSPHATE

As a part of a systematic study of the conformations of nucleic acid constituents, we have now taken up the X-ray crystal structure analysis of the mononucleotides—deoxyguanosine-5' phosphate and guanosine-5' phosphate. Conformational differences between the two due to the additional hydroxyl group on C(2') of the ribose, the nature of base stacking, tautomeric form and hydrogen bonding will be of interest to the stereochemistry of nucleic acids.

Crystals of sodium salts of deoxyguanosine-5' phosphate and guanosine-5' phosphate were grown

Attempts to crystallise from acetone solutions, normally found successful in the case of other mononucleotides<sup>1-3</sup> yielded only a gel like precipitate.

Paper chromatography carried out by descending technique on Whatman No. 1 paper using the solvent isopropyl alcohol, concentrated ammonia and water in the ratio 7:2:1 confirmed the crystals to be dGMP-5' and GMP-5'.

Rotation, Weissenberg and precession photographs were taken using CuK $\alpha$  radiation. The crystal data of both these compounds are given in Table I. Densities were measured by flotation method using carbon tetrachloride, bromoform mixtures.

TABLE I

	dGMP-5'Na <sub>2</sub>	GMP-5 Na
Formula	C <sub>10</sub> H <sub>12</sub> N <sub>6</sub> O <sub>7</sub> P Na <sub>2</sub> 4H <sub>2</sub> O	C <sub>10</sub> H <sub>13</sub> N <sub>6</sub> O <sub>8</sub> PNa 9H <sub>2</sub> O
M.W.	391.2	384.2
System	Monoclinic	Orthorhombic
<i>a</i>	16.002 Å	22.466 Å
<i>b</i>	10.730 Å	21.425 Å
<i>c</i>	5.575 Å	9.023 Å
$\beta$	101.9°	..
<i>V</i>	936.65 Å <sup>3</sup>	4343.07 Å <sup>3</sup>
<i>d</i> <sub>obs</sub>	1.64 gm/cc	1.67 gm/cc
<i>d</i> <sub>cal</sub>	1.64 gm/cc	1.66 gm/cc
<i>Z</i>	2	8
$\mu$ (CuK $\alpha$ )	1.5418 Å	1.5418 Å
Systematic absences	<i>oko</i> ( <i>k</i> = odd)	<i>hoo</i> ( <i>h</i> = odd) <i>oko</i> ( <i>k</i> = odd) <i>ool</i> ?
Space group	P2 <sub>1</sub>	P2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub> or p2 <sub>1</sub> 2 <sub>1</sub> 2

by slow diffusion of alcohol into aqueous solutions of the compounds. Needle like crystals as long as 5 to 6 mm could be grown in about two weeks.

The authors thank Prof. P. S. Narayanan for his kind interest in this work. They thank Dr. T. M. Jacob and Mr. A. S. Gopalakrishnan of Biochemistry

Department of the Institute for performing the paper chromatography test. One of us (T. P. S.) thank the UGC authorities for awarding Junior Fellowship during the period of this work.

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### VIBRATIONS OF A NON-ISOTROPIC TRAPEZOIDAL PLATE

#### ABSTRACT

This paper deals with the vibration of an isosceles trapezoidal plate of non-isotropic material resting on elastic foundation.

DE<sup>1,2</sup> recently solved the problems of the vibrations of rectangular, circular, elliptical and right-angled triangular plates of non-isotropic material. In this paper, the vibrations of an isosceles trapezoidal plate of a particular type of anisotropic material with base angle 45°, resting on elastic foundation is considered. The exact solution for the frequency is given when the plate is simply supported on all edges.

We assume that the material of the plate has three planes of symmetry with respect to its elastic properties. We choose these planes as the coordinate planes. The  $xy$ -plane is along the middle plane of the plate in the unstrained condition. We assume that the plate is made of such a material that the elements perpendicular to the middle plane remain perpendicular to it after being strained.

The differential equation for the vibration of the plate (for concrete slabs) can be written as<sup>2</sup>

$$D_x \frac{\partial^4 w}{\partial x^4} + 2 \sqrt{D_x D_y} \frac{\partial^4 w}{\partial x^2 \partial y^2} + D_y \frac{\partial^4 w}{\partial y^4} + \rho \frac{\partial^2 w}{\partial t^2} + \beta w = 0, \quad (1)$$

where

$$D_x = E_x' h^3/12, \quad D_y = E_y' h^3/12,$$

$E_x'$ ,  $E_y'$  are elastic constants,  $h$  denotes the thickness of the plate,  $\rho$  is the density of the material of the plate and  $\beta$  is the modulus of the elastic foundation.

Assuming  $w = W(x, y)e^{ipmt}$  ( $p_m$  is the angular frequency) and substituting

$$x = x' D_x^{1/4}, \quad y = y' D_y^{1/4},$$

we have from

$$\left( \frac{\partial^2}{\partial x'^2} + \frac{\partial^2}{\partial y'^2} \right) \left( \frac{\partial^2}{\partial x'^2} + \frac{\partial^2}{\partial y'^2} \right) w - \gamma_m^2 w = 0, \quad (2)$$

where

$$\gamma_m^2 = \rho p_m^2 - \beta.$$

We consider a trapezoidal plate of height 'a' whose parallel sides are  $2a$  and  $4a$  respectively (Fig. 1). We consider here that all the edges of the plate are simply supported.

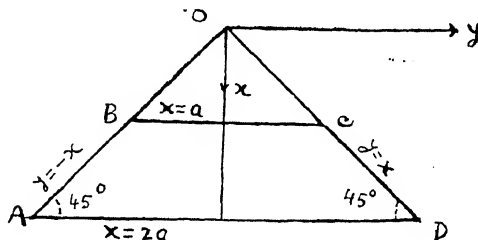


FIG. 1

The boundary conditions are

$$\begin{aligned} W &= 0 \text{ for } x = a, 2a \text{ and for } x \pm y = 0 \\ \frac{\partial^2 W}{\partial x^2} &= 0 \text{ for } x = a, 2a, \\ \frac{\partial^2 W}{\partial y^2} &= 0 \text{ for } x \pm y = 0 \end{aligned} \quad (3)$$

where

$$\frac{\partial}{\partial \eta_{1,2}} = \frac{1}{\sqrt{2}} \left( \frac{\partial}{\partial x} \pm \frac{\partial}{\partial y} \right).$$

These conditions in the transformed coordinates can be written as

$$\begin{aligned} W &= 0 \text{ for } x' = a', 2a' \text{ and for } x' \pm y' = 0, \\ \frac{\partial^2 W}{\partial x'^2} &= 0 \text{ for } x' = a', 2a', \\ \frac{\partial^2 W}{\partial y'^2} &= 0 \text{ for } x' \pm y' = 0 \end{aligned} \quad (4)$$

where

$$\frac{\partial}{\partial \eta'_{1,2}} = \frac{1}{\sqrt{2}} \left( \frac{\partial}{\partial x'} \pm \frac{\partial}{\partial y'} \right).$$

The boundary conditions can be satisfied identically by choosing a series of the form

$$W = \sum_m A_m \left( \sin \frac{2m\pi x'}{a'} \sin \frac{m\pi y'}{a'} - \sin \frac{2m\pi y'}{a'} \times \sin \frac{m\pi x'}{a'} \right), \quad (5)$$

where  $A_m$  is a constant and  $m$  is a positive integer. Substituting (5) in (2), we have the frequency equation

$$\left[ 9 \left( \frac{m\pi}{a'} \right)^4 - \gamma_m^2 \right] = 0 \quad (6)$$

i.e.,

$$p_m^2 = \frac{1}{\rho} \left[ 9 D_x \left( \frac{m\pi}{a} \right)^4 + \beta \right]. \quad (7)$$

For  $m = l$ , we have the frequency of the gravest mode of vibration.

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# X-RAY STUDY OF THE STRUCTURE AND IONIC CONFIGURATION OF $\text{CoMnGaO}_4$

## ABSTRACT

A new compound  $\text{CoMnGaO}_4$  is synthesized and its crystal structure has been determined by powder method. It is found that this compound crystallizes in cubic spinel structure with  $a = 8.30 \pm 0.02$  Å. The oxidation state of manganese and cobalt was determined X-ray spectroscopically as two and three respectively. The ionic configuration of this spinel is found to be  $\text{Ga}^{+3} [\text{Mn}^{+2} \text{Co}^{+3}] \text{O}_4^{2-}$ .

**Keywords:** Spinel Structure, X-ray absorption spectroscopy,  $\text{CoMnGaO}_4$ .

THE oxidic spinels have wide utility in industries because of their remarkable electrical and magnetic properties, which depend upon the valency and the crystallographic positions of cations in this paper we have determined the charge distribution and the site distribution of the cations. In the spinel  $\text{CoMnGaO}_4$  using X-ray spectroscopic and X-ray diffraction techniques because of the specificity of the method (Kulkarni *et al.*, 1971).

It appears that there is no report of the above compound in the literature.

The compound  $\text{CoMnGaO}_4$  was prepared by intimately mixing  $\text{MnO}$ ,  $\text{Co}_2\text{O}_3$  and  $\text{Ga}_2\text{O}_3$  of analar grade in molar ratio 2 : 1 : 1. The mixture was heated in an electrically operated furnace at  $900^\circ\text{C}$  for 100 hours in a Pt boat. From the powder pattern taken on a 57.3 mm Debye Scherrer Camera using filtered Cobalt  $K_\alpha$  radiation, formation of the compound was taken to be complete when the lines due to the reacting oxides are absent. The observed  $1/d^2$  values for various reflections along with the calculated  $1/d^2$  values and observed and calculated intensities of the various lines are included in Table I. The detailed procedure for calculations of intensities and X-ray spectroscopic determination of oxidation states of cations have already been reported in our earlier paper (Bhalerao *et al.*, 1973). The observed intensity ratios show that  $\text{Ga}^{+3}$  ions are at the tetrahedral (A) sites. In order to determine the oxidation state of the cations in this spinel we have studied the K absorption discontinuities of manganese and cobalt in pure metals, in some of their compounds of well-known oxidation states and in the above spinel. Our X-ray spectroscopic results for manganese and cobalt ions are given in Table III and II respectively. We have not studied the absorption spectra of gallium in the spinel because it has most stable valency of three (Remy, 1956).

It has already been found out from observed X-ray intensity ratios that  $\text{Ga}^{+3}$  ions occupy the A

TABLE I

X-ray diffraction data of the spinel  $\text{CoMnGaO}_4$   
 $a = 8.30 \pm 0.02$  Å

hkl	$1/d^2$		Intensity	
	Obs.	Cal.	Obs.	Cal.
111	0.0446	0.0435	<5	<3
220	0.1172	0.1160	40	34.5
311	0.1615	0.1595	100	100
222	0.1770	0.1740	5	6.8
400	0.2366	0.2320	20	13.5
422	0.3512	0.3480	15	12.3
511	0.3924	0.3915	35	30.9
440	0.4645	0.4640	50	42.4
533	0.6208	0.6235	10	11.6
444	0.6943	0.6960	<5	<3
711	0.7461	0.7395	15	10.0
642	0.8115	0.8120	10	8.5
731	0.8569	0.8555	20	19.8
800	0.9245	0.9280	10	11.5
751	1.0847	1.0875	15	13.6
555				4.5

TABLE II

Absorber	Valency	Wavelength x.u.	Chemical shift x.u.
Co metal (Cauchois and Hulubei, 1947)	..	1604.87	..
Co metal (Present work)	..	1604.9	..
Co O	2	1602.9	2.0
CoSO <sub>4</sub>	2	1602.6	2.3
CoCl <sub>2</sub>	2	1602.5	2.4
Co <sub>2</sub> O <sub>3</sub>	3	1601.3	3.6
Co[(NH) <sub>5</sub> Cl <sub>2</sub> ]NO <sub>3</sub>	3	1601.5	3.4
CoMnGaO <sub>4</sub>	..	1601.6	3.3

TABLE III

Absorber	Valency	Wavelength x.u.	Chemical shift x.u.
Mn metal (Cauchois and Hulubei, 1947)	.. ..	1892.54	..
Mn metal (Present work)	.. ..	1892.6	..
MnCL <sub>2</sub> : 4H <sub>2</sub> O	.. 2	1890.9	1.7
MnO	.. 2	1890.8	1.8
MnCO <sub>3</sub>	.. 2	1891.1	1.5
Mn <sub>2</sub> O <sub>3</sub>	.. 3	1888.9	3.7
Mn <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	.. 3	1889.1	3.5
CoMnGaO <sub>4</sub>	.. ..	1891.0	1.6

sites which is in accord with the calculated site preference energies for Ga<sup>+3</sup> (15.4 K Cal/g at wt) and Mn<sup>+2</sup> (14.7 K Cal/g at wt) at A sites given by Miller (1959). Thus possible valence distribution for this spinel are

- (i) Ga<sup>+3</sup>[Mn<sup>+2</sup>Co<sup>+3</sup>]O<sub>4</sub><sup>-2</sup> and  
(ii) Ga<sup>+3</sup>[Mn<sup>+3</sup>Co<sup>+2</sup>]O<sub>4</sub><sup>-2</sup>

From the observed chemical shifts of manganese and cobalt ions given in Tables III and II it is found that manganese ions are in divalent and cobalt ions are in the trivalent state in this spinel. The lattice parameter 'a' may be calculated empirically by using Mikheev's (1955) formula using the values of ionic radii given by Arhens (Azroff, 1960). It is found that the observed lattice parameter 8.30 Å fits well with the calculated lattice parameter 8.29 Å for the model containing Mn<sup>+2</sup> ions at the B sites. Hence we may write the ionic structure of this spinel as Ga<sup>+3</sup>[Mn<sup>+2</sup>Co<sup>+3</sup>]O<sub>4</sub><sup>-2</sup>.

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MAGNETIC SUSCEPTIBILITY OF THE  $>\text{CH}_2$   
GROUP IN HOMOLOGOUS SERIES OF  
ALKYL ARYL SULPHIDES

DURING our systematic study of the diamagnetic susceptibilities of organic compounds<sup>1-4</sup>, we determined the magnetic susceptibilities of some *p*-alkylphenyl methyl sulphides and alkyl aryl sulphides, and the methylene group contribution in these compounds is reported here.

The methylene group contribution in the *p*-alkylphenyl methyl sulphides (*p*-alkyl=ethyl, isopropyl and *t*-butyl) is recorded in Table I. For comparison the  $\text{CH}_2$  increment of the corresponding

bility of any interaction between the alkyl and methylthio groups through the benzene ring even though sulphur may function as an electron-acceptor when an electron-releasing substituent is present para to the methylthio group<sup>6,7</sup>.

The magnetic susceptibilities of benzyl methyl sulphide, ethyl phenyl sulphide, ethyl *p*-tolyl sulphide, isopropyl phenyl sulphide and *n*-propyl phenyl sulphide and the  $\text{CH}_2$  increments are recorded in Table II. The low methylene group increment from methyl phenyl sulphides to ethyl phenyl sulphides seems to be very characteristic, but the cause of this is not clear.

TABLE I

Compound	$\chi_M^*$	$\chi > \text{CH}_2$		$\chi_M$	$\chi > \text{CH}_2$	
<i>p</i> -C <sub>2</sub> H <sub>5</sub> ·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	105·2	11·8	}	C <sub>6</sub> H <sub>5</sub> ·C <sub>2</sub> H <sub>5</sub>	77·2	11·6
<i>p</i> -CH <sub>3</sub> ·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	93·4			C <sub>6</sub> H <sub>5</sub> ·CH <sub>3</sub>	65·6	
<i>p</i> -(CH <sub>3</sub> ) <sub>2</sub> CH·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	117·6	12·4	}	C <sub>6</sub> H <sub>5</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	89·5	12·3
<i>p</i> -C <sub>2</sub> H <sub>5</sub> ·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	105·2			C <sub>6</sub> H <sub>5</sub> C <sub>2</sub> H <sub>5</sub>	77·2	
<i>p</i> -(CH <sub>3</sub> ) <sub>3</sub> C·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	129·0	11·4	}	C <sub>6</sub> H <sub>5</sub> C(CH <sub>3</sub> ) <sub>3</sub>	101·1	11·6
<i>p</i> -(CH <sub>3</sub> ) <sub>2</sub> CH·C <sub>6</sub> H <sub>4</sub> ·SCH <sub>3</sub>	117·6			C <sub>6</sub> H <sub>5</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	89·5	

\*  $\chi_M$  is given in units of  $-10^{-6}$  emu.

TABLE II

Compound	$\chi_M$	$\chi > \text{CH}_2$
$\text{C}_6\text{H}_5\text{SC}_2\text{H}_5$	93.6	10.8
$\text{C}_6\text{H}_5\text{SCH}_3$	82.8	
<i>p</i> - $\text{CH}_3 \cdot \text{C}_6\text{H}_4 \cdot \text{SC}_2\text{H}_5$	104.3	10.9
<i>p</i> - $\text{CH}_3 \cdot \text{C}_6\text{H}_4 \cdot \text{SCH}_3$	93.4	
$\text{C}_6\text{H}_5\text{SCH}_2\text{CH}_2\text{CH}_3$	105.1	11.5
$\text{C}_6\text{H}_5\text{SC}_2\text{H}_5$	93.6	
$\text{C}_6\text{H}_5\text{SCH}(\text{CH}_3)_2$	105.2	11.6
$\text{C}_6\text{H}_5\text{SC}_2\text{H}_5$	93.6	
$\text{C}_6\text{H}_5\text{CH}_2\text{SCH}_3$	94.3	11.5
$\text{C}_6\text{H}_5\text{SCH}_3$	82.8	

alkylbenzenes (values from Angus *et al.*<sup>5</sup>) is also recorded. The close agreement of the methylene group contribution between the *p*-alkylphenyl methyl sulphides and the alkylbenzenes rules out the possi-

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ISOLATION OF "TRICHOTETROL"—A NEW  
TETRAHYDROXY PENTACYCLIC TRITERPENE  
FROM *TRICHOSANTHES BRACTEATA*  
(CUCURBITACEAE). LINN. VOIGHT. SYN. T.  
PALMATA (ROX. )

THE root of the plant, *Trichosanthes bracteata* is used in lung diseases of cattle, for treatment of carbuncles, and for headache<sup>1</sup>.

As the presence of a bitter substance was reported<sup>2</sup> from this plant long ago, a reinvestigation of the root has now been taken up. Powdered dry root of the drug (10 kg) in portions was initially extracted with hot petroleum-ether and then with hot alcohol. The combined alcoholic extract was concentrated and kept overnight. Some substance along with wax separated. It was filtered off and the mother liquor was concentrated and kept in a refrigerator overnight. More material then separated out along with wax. It was filtered and the total waxy residue thus obtained was treated with hot high boiling (60–80°) Petroleum-ether to dissolve the wax. On keeping the petrol solution the undissolved substance settled down. It was filtered and washed several times with hot petroleum ether. The crude substance (2.5 g) thus obtained was found to be insoluble in ether, chloroform and benzene. It was purified by repeated crystallisation from a 1:1 mixture of methyl alcohol-ethyl acetate (the substance dissolved only after refluxing for a long time). Three repeated crystallisations from the above mixture of solvents gave a crystalline substance, m.p. 289 to 291°. Its Infrared spectrum in nujol displayed a broad band around 3480 (OH groups), a twin band at 1380 due to the gem dimethyl group, and a sharp band at 1160 cm<sup>-1</sup> probably due to an ether linkage. This spectrum also showed prominent bands at 830 and 885 cm<sup>-1</sup>.

**Acetylation.**—The substance (1 g) obtained above was treated with acetic anhydride (10 ml) and pyridine (3 ml) and kept at 100° till the substance completely dissolved. The reaction mixture was kept overnight and poured into crushed ice with stirring. The solid separated was filtered, washed with dilute hydrochloric acid followed by distilled water. It (1 g) was then crystallised from methanol. m.p. 175–176°. Found : C, 69.37; H, 8.85; Acetyl value 27.4. C<sub>30</sub>H<sub>48</sub>O<sub>5</sub> requires C, 69.48; H, 8.59; 4 Acetyl 26.22%. Its I.R. spectrum in methylene chloride showed absence of OH. However it showed a sharp new band at about 1735 cm<sup>-1</sup> (ester carbonyl), a twin band around 1380 (gem dimethyl) and another band at 1160 cm<sup>-1</sup> probably due to ether linkage. Its 100 MC NMR spectrum in CDCl<sub>3</sub> showed

four sharp signals around δ 1.7 corresponding to four acetyl groups (12 H; each of 3-proton intensity as singlets) and a number of peaks between δ 0.7 to 1.4 corresponding to tertiary methyls.

**Deacetylation.**—The acetyl derivative (0.5 g) was refluxed with sodium hydroxide (60 ml 5%) for 8 hours. It was then cooled and diluted with water and the product was crystallised from methanol-ethyl acetate (1:1) mixture (m.p. 293–295°, 0.3 g). Found : C, 73.62; H, 10.34; active hydrogen 0.41. C<sub>30</sub>H<sub>48</sub>O<sub>5</sub> requires C, 73.73; H, 9.90%).

The original compound and its acetyl derivative gave colouration with tetranitromethane thereby indicating the presence of hindered double bond. The acetyl derivative gave a positive Libermann-Burchard test (pinkish to intense blue to green). The acetyl derivative did not undergo selenium dioxide oxidation probably due to the presence of a hindered double bond.

From the above findings it is suggested that the compound isolated is a new neutral pentacyclic triterpene named as trichotetrol having the molecular formula C<sub>30</sub>H<sub>48</sub>O<sub>5</sub> and containing only four hydroxyl groups. The fifth oxygen may be present as an oxide linkage.

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## A NEW HOST FOR BACTERIAL LEAF BLIGHT PATHOGEN OF RICE

BACTERIAL leaf blight disease of rice has been reported to occur on several hosts belonging to the families, Gramineae<sup>1,2,3,7</sup> and Cyperaceae<sup>2</sup> in nature. Two species of *Leptochloa*, *L. chinensis* and *L. panacea*, *Zizania leptocarpa*<sup>3</sup>, two species of *Leersia*, *L. hexandra*<sup>6</sup>, *L. japonica*, *Phalaris arundinacea*, *Phragmites communis*, *Isachne globosa* and *Setaria viridis*<sup>7</sup> were, however, found to be susceptible to *Xanthomonas oryzae* under artificial inoculation tests. Further investigations were undertaken to search for new hosts of this pathogen.

Twenty-one graminaceous weeds commonly found in and around rice fields, were collected from Central Rice Research Institute Farm and were maintained in earthen pots alongwith a susceptible rice cultivar T (N) 1 during *kharif* season, 1973. The plants were inoculated by clipping the leaves

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with a pair of scissors previously dipped into a bacterial cell suspension (*ca.*  $10^8$  cells/ml)<sup>1</sup> prepared from a 48 hr old culture of a virulent isolate of *X. oryzae* grown on potato sucrose agar medium. Controls were maintained by clipping the leaves with a pair of scissors dipped into sterile distilled water. The disease development was observed periodically and the lesion length was measured on 15th day after inoculation.

infected leaves and identified as *X. oryzae* and pathogenicity was confirmed on their respect hosts. Cross inoculation tests proved easy cross over from one host to the other.

Our findings are in conformity with those of R and Kauffman<sup>6</sup> who reported the susceptibility of *L. hexandra* to *X. oryzae* under artificial conditions. In the present study *P. scorbiculatum* was found to be a new addition to the host range of *X. oryzae*.

TABLE I

*Bacterial leaf blight development on two graminaceous weeds as compared to rice cultivar T(N) 1*

Host	Symptom initiation (days)	Mean leaf length (cm)	Mean lesion length (cm)	% infected	Cross inoculation Isolate from		
					<i>O. sativa</i>	<i>L. hexandra</i>	<i>P. scorbiculatum</i>
<i>O. sativa</i> [T(N) 1]	4	30.9	25.5	82.5	+++	+++	+++
<i>L. hexandra</i>	8	12.6	1.9	15.1	+	+	+
<i>P. scorbiculatum</i>	6	7.7	6.8	88.3	++	++	++

+ Represents the disease intensity.

Among the grass weeds tested, *L. hexandra* and *Paspalum scorbiculatum* were found to be susceptible to this disease. The initiation of the disease symptom was delayed in *L. hexandra* with slow downward development of necrotic lesion when compared to *P. scorbiculatum* and T(N) 1. The lesion length was less in *P. scorbiculatum* when compared to T(N) (Table I). The inoculated leaves of *P. scorbiculatum* showed typical yellow discoloration of the tissue which preceded the lesion development without any well defined margin (Fig. 1). Control leaves did not exhibit any such symptom.

Natural incidence of this disease was however, observed in the field on any of these two hosts.

We are grateful to Dr. S. Y. Padmanabhan, Director, for his interest and encouragement and thank to Dr. N. K. Chakrabarty, Head, Plant Pathology, providing the facilities. Botanical Survey of India Calcutta and Sri. N. K. C. Pattnaik are acknowledged for identifying the grass weeds.

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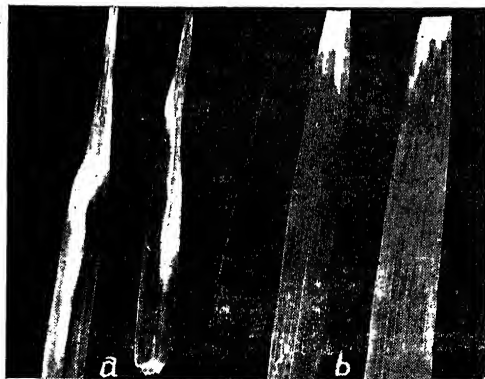


FIG. 1. Symptoms on the inoculated leaves of *P. scorbiculatum* (a) and *L. hexandra* (b).

Microscopic examination of the diseased leaves revealed typical bacterial oozing from the infected tissues. The pathogen was isolated from the

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# LEPTOSPHAERULINA LEAF SPOT OF COW-PEA, A NEW RECORD TO INDIA

A SEVERE leaf spot disease of cow-pea was noticed in the fields of Agricultural College, Dharwar, during July 1973. The microscopic examination of the infection spots revealed the presence of uniloculate ascostroma containing bitunicate asci not intermingled with sterile threads, on the basis of which it was diagnosed as a species of *Leptosphaerulina* Mc Alp. As there is no report of the species of *Leptosphaerulina* causing leaf spots on cow-pea in India, it being a new report, a brief description of the symptoms of disease is given here. However, Graham and Luttrell (1961) have obtained infection on *Vigna sinensis* (Torner) Savi by artificial inoculation with the culture of *Leptosphaerulina trifolii* (Rost.) Petr.

Symptoms (Fig. 1) were seen only on leaves. Small circular spots were produced on the leaves and particularly along the margin of the leaves. The centre of the spot was yellowish-white with dark-brown margin. Further, the spots became irregular due to expansion and coalescing. Black small spherical bodies were prominently seen in the necrotic area. Later, the infected leaves dried up and fell down.

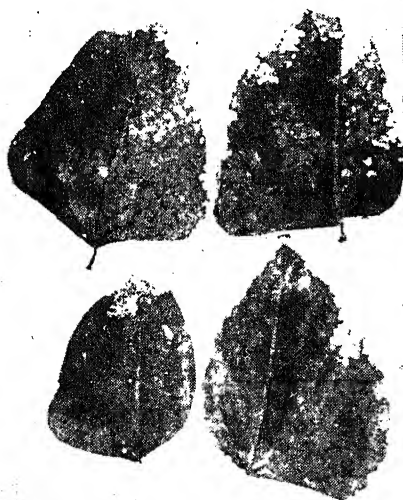


FIG. 1. Leaves showing the symptoms of the disease.

Graham and Luttrell (1961) described various species of *Leptosphaerulina*, and a comparative study was carried out with the species of *Leptosphaerulina*, which revealed that the present species of *Leptosphaerulina* resembled *Leptosphaerulina trifolii* on the basis of morphological characters and

dimensions of ascocarps, asci, and ascospores. Hence, it was diagnosed as *L. trifolii*.

The material of the above specimen is deposited at the Herbarium Cryptogamae Indiae Orientalis, I.A.R.I., New Delhi-12, No. 31638.

The authors express their sincere thanks to Dr. R. S. Deshpande, Professor of Plant Pathology, College of Agriculture, Dharwar, for the encouragement.

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## KETO ACID CHANGES DURING FRUIT-SETTING IN *CICER ARIETINUM*

KETO acids occupy a unique position in plant metabolism. Inorganic nitrogen assimilation is directly linked with these substances<sup>1</sup>. These are also utilized for the synthesis of carbohydrates<sup>2</sup>. In spite of their key role in intermediary plant metabolism very few attempts have been made to study their changes during flowering and fruit-setting periods<sup>3-4</sup>. The present study shows various changes in keto acid composition during fruit-setting period.

*Cicer arietinum* plants cultivated in the University campus have been selected for this study. Keto acids are extracted as their 2,4-dinitrophenyl hydrazones (2,4-DNP's) as described elsewhere<sup>3-5</sup>. Paper and thin layer chromatographic techniques have been employed for the separation of these metabolites. Amounts of keto acids have been calculated in terms of 2,4-DNP of  $\alpha$ -ketoglutaric acid using Klett photoelectric colorimeter.

Relative position of different keto acids on chromatograms as noticed in the present study are shown in Fig. 1. Amino acid changes are also observed besides keto acids in mature leaves and immature fruits at the time of fruit-setting. The total concentration of keto acids is about four and a half fold more in leaves compared to fruits (Table I). Similarly, higher levels of amino acids are also encountered in leaves (Table II). The concentration of keto acids is much greater than amino acids. Glyoxylic acid, pyruvic acid, oxaloacetic acid (OAA-F) and  $\alpha$ -ketoglutarate ( $\alpha$ -KGA) are the known keto acids synthesized in this plant. Urea also appears to be synthesized and accumulated in mature leaves. These substances, however, do not appear in immature fruits. Besides these compounds, a number of unknown hydrazone

TABLE I

Changes in keto acids (mg/5 g fresh weight) in terms of 2, 4-DNP of  $\alpha$ -KGA in leaves and immature fruits during fruit-setting (—absent)

Plant Parts	Glyoxylic acid	Pyruvic acid	OAA(F)	$\alpha$ - KGA	Urea (F)	Un <sub>2</sub>	Un <sub>3</sub>	Un <sub>8</sub>
1. Mature leaves	4.0	3.2	3.6	2.0	8.0	7.6	3.2	0.8
2. Immature fruits	Trace	..	..	..	..	7.2	..	Trace

TABLE II

Changes in amino acids (mg/5 g fresh weight) in terms of glycine in leaves and immature fruits during fruit-setting

Plant Parts	Valine	Alanine	Glutamic acid	Aspartic acid	Glycine & Serine
1. Mature leaves	.. Trace	0.133	0.533	0.266	0.133
2. Immature fruits	.. Trace	Trace	0.266	0.200	..

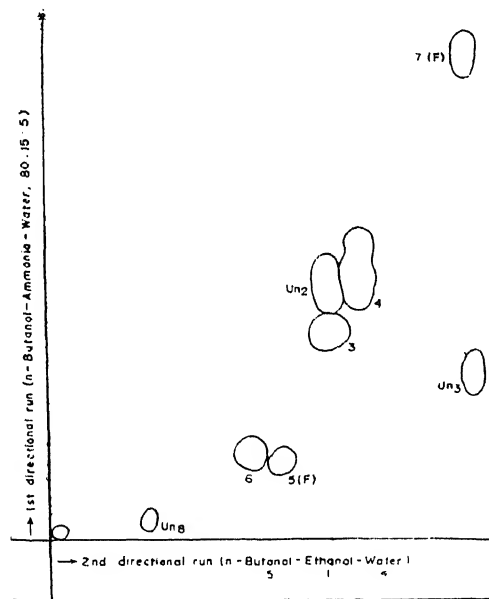


FIG. 1. *C. arietinum*—Diagrammatic representation of chromatographic separation of keto acids. 7(F)—Urea; Un<sub>2</sub>, Un<sub>3</sub>, Un<sub>8</sub>—Unidentified spots; 3—Glyoxylic acid; 4—Pyruvic acid; 5(F)—Oxaloacetic acid; 6— $\alpha$ -Ketoglutaric acid; (F)—Fast moving isomer.

spots, e.g., Un<sub>2</sub>, Un<sub>3</sub> and Un<sub>8</sub> have been also noticed.

Only five amino acid spots have been noticed compared to a large number of keto acids in these

plant parts (Table II). Alanine, glutamic acid, aspartic acid, glycine, serine and valine have been observed in mature leaves. The concentrations of the first four compounds are found to be low while the last one could be observed only in trace amount. In immature fruits, a little amount of glutamic acid and aspartic acid could be seen. Valine and alanine appear in traces while glycine and serine could not be detected.

From the overall pattern of distribution of different metabolic components in *C. arietinum*, it is evident that there is a direct correlation between active growth period and rapid utilization of these substances. Since the process of growth becomes very slow, the concentration of keto acids increases considerably, resulting in the accumulation of these substances in mature leaves. The higher level of metabolites in mature leaves clearly shows that the requirement of these substances diminishes with ageing. Similar observation has been reported earlier in groundnut seedlings<sup>6</sup> and this is also evident from the studies on *Bauhinia purpurea*<sup>5</sup>.

In the case of immature fruits, however, as the keto acid metabolites are being constantly used up for the development process, they are present in very little amounts. This is in agreement with the high metabolic rate in this part compared to the sluggish activities in the mature leaf tissues.

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#### A MODIFIED SCHIFF REAGENT FOR USE IN FEULGEN REACTION

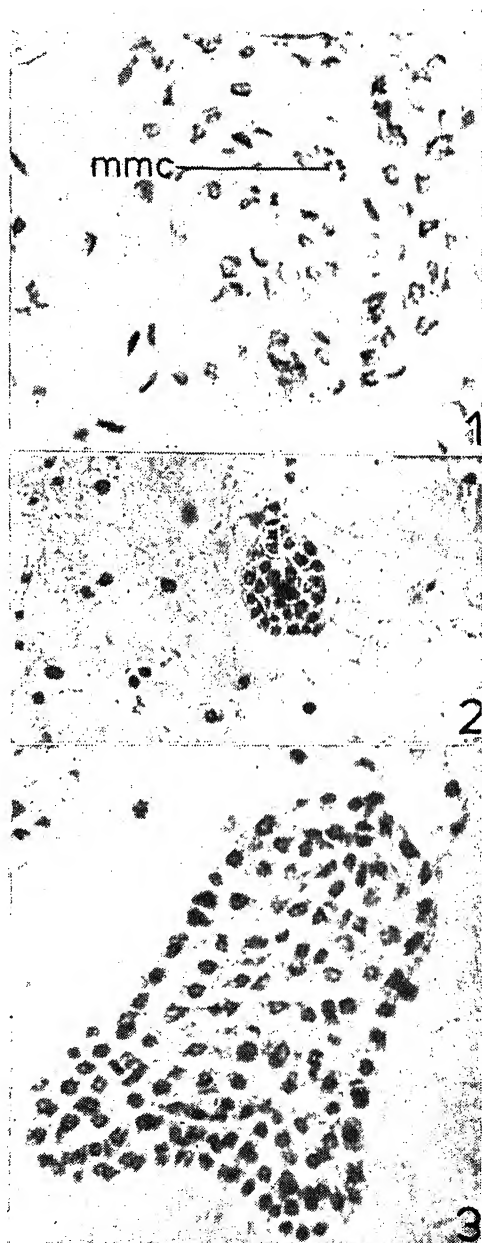
THE Feulgen reaction is a well-known technique for localising deoxyribonucleic acid (DNA). Since its development by Feulgen and Rossenbeck<sup>1</sup>, various modifications of the methodology have been suggested. The procedure of preparing the Schiff reagent has been varied. The modifications suggested by de Tomasi<sup>2</sup>, Lillie<sup>3</sup> and Longley<sup>4</sup> are being widely used for the localisation of DNA in the Feulgen nuclear technique. However, with the above Schiff reagents, staining was not satisfactory in embryological preparations of plants like *Linaria bipartita*, *Lindenbergia indica*, and *Phlebo-phyllum kunthianum* and a few others. Lillie's modification of the Schiff reagent gave intense staining in the PAS technique in all these plants.

A combination of methods suggested by de Tomasi and Lillie was attempted, which gave a very intense staining for DNA. This involves the preparation of the Schiff reagent as follows: Dissolve 1 gm of new fuchsin (E. Merck Ag. Darmstadt, C.I. Nr. 42520) in a standard flask containing 100 ml boiling, distilled water. Shake thoroughly. Cool to 50° C. Add 10 ml of 1/N hydrochloric acid, and 2 gm of potassium metabisulphite. Shake thoroughly. Close the vessel tightly and store it in a refrigerator for 24 hours. Decolourise the solution by shaking with 0.5 gm of activated charcoal and filter until the filtrate is colourless. The pH of the reagent would be 3.0; store it in a refrigerator.

In the case of *Linaria bipartita* materials fixed in either acetic-alcohol or neutral formalin and subsequently hydrolysed for 12 minutes in 1 N hydrochloric acid at 60° C and treated with the above modified Schiff reagent for 3 hours, staining was highly satisfactory (Figs. 1-3).

In the preparation of the Schiff reagent described above the quantities of new fuchsin and the bleach (potassium metabisulphite) taken were the same as those used by Lillie (Lillie used sodium

metabisulphite). The method employed in preparation was that of de Tomasi. The amount of 1 N hydrochloric acid used, however, was 10 ml instead of 15 ml used by Lillie. The pH of the reagent is 3.0 and it is same as that recommended by Itikawa and Ogura<sup>5</sup>. These authors have shown



Figs. 1-3. Fig. 1. Ls. ovule showing megaspore mother cell,  $\times 800$ . Fig. 2. A globular embryo and part of the endosperm,  $\times 433$ . Fig. 3. Heart-shaped embryo,  $\times 663$ . (mmc, megaspore mother cell).

that the optimum pH for the best Feulgen staining is 3.0. Other investigators<sup>2-4,8</sup> have also found intense staining with Schiff reagent having this particular pH. These investigators have used 1N sodium hydroxide to adjust the pH to 3.0.

I am grateful to Professor H. Y. Mohan Ram for suggestions and Dr. S. P. Bhatnagar for encouragement. I thank Dr. M. R. Vijayaraghavan for guidance. The award of a Junior Research Fellowship by the Council of Scientific and Industrial Research is acknowledged.

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#### OCCURRENCE OF SYMBIOSIS IN SOME NON-LEGUMINOUS PLANTS

EVIDENCE have been recorded for the occurrence of bacterial symbionts in many plant genera other than those of *Leguminosae*<sup>1-4,7</sup>. In these reports, structures like bacterial colonies have been observed in the free hand sections of slightly underground stems as well as aerial stems of some non-Leguminous plants. The plants reported for the bacterial symbionts are *Scirpus squarrosus*<sup>6</sup>, *Cyperus papyrus*<sup>7</sup>, *C. rotundus*<sup>1</sup>, *Amaranthus oleraceus*<sup>1</sup>, *A. viridis*, and *A. gangeticus*<sup>5</sup>. In the present note, one of the above species from *Amaranthaceae* is confirmed for the presence of bacterial symbionts. In addition record of three new species showing the bacterial symbionts in aerial as well as in slightly under ground stem portions is made. It also includes the detailed studies of isolated bacterium from the plants of *Amaranthus spinosus*.

Various weed species of plants were collected from the campus and Botanical Garden of the Institute. These are *Amaranthus spinosus* L., *A. polygamus* L., *Achyranthus aspera* L., *Digera arvensis* Forsk., *Datura metel* L., *Solanum xanthocarpum* S. & W., *Physalis minima* L., *Ocimum canum* L., *Boerhaavia diffusa* L. and *Evolvulus alsinoides* L.

The underground stems and serial stems of each plant were separated from the remaining portions. Free hand sections were prepared from both the stem portions and were suspended in 1/1000 aqueous solution of HgCl<sub>2</sub> to disinfect the surface of the sections in separate bottles for 2 min. and were placed in clean sterile glass slides. All the sections were examined microscopically at 1000 × magnification after removal of chlorophyll by ethanolic fixation and application of Gram's staining technique<sup>3</sup> (Hucker modification). Almost all the sections of the plants *Amaranthus spinosus* L., *A. polygamus* L., *Achyranthus aspera* and *Digera arvensis* Forsk. showed the presence of red-colored dots in the sections, while the sections from other genera did not show the presence of the structure. The sections of leaf, petiole and roots were taken from the above four species and studied. In all such sections red-colored dots were absent indicating the absence of bacterial symbionts.

The observation was confirmed by crushing the various disinfected unstained fresh sections on clean sterile glass slide aseptically, and then fixed by heat, for staining and of Gram's staining technique<sup>3</sup>. The only previous positive cases showed the presence of irregular, Gram negative rods which were capsulated. However, flagella staining did not show flagella.

The work was completed by the isolation of the bacterium involved in one species. For this only one species *Amaranthus spinosus* was selected and free hand sections were prepared from the aerial and slightly under ground stems which were disinfected and placed in sterile distilled water tubes for 2 hr at room temperature and was plated. Then usual poured plate technique was performed in Mannitol Agar with Yeast extract (YMA) and YMA with added Congo red<sup>2</sup>. The plates were incubated at room temperature. After 72 hr of incubation, the plates were checked for the growth. All the plates gave same type of colonies on the above plates indicating that the bacterium is of the same type, which was also confirmed by observation on morphological and physiological characteristics. The cultural, morphological and physiological characteristics of the bacterium isolated from the *Amaranthus spinosus* are as below: Colonies on YMA and YMA-Congo red media—big, circular, smooth surface, entire margin, butyrous and glistening white. The colonies become slight pink after 6 days of incubation. Morphology and physiological characteristics—short rods, measuring from 1.1–3.0 × 0.5–0.9 microns, Gram-negative, motile with peritrichous flagella, capsulated, no endospore, non-acid-fast and contain fat globules, occur singly

or in short chains. Chemical characteristics are as follows.

Starch hydrolysed, casein digested, tributyrin hydrolysed gelatin not liquefied, milk peptonized and litmus reduced, nitrates reduced to nitrites, hydrogen sulfide and indole not produced, V.P. and M.R. tests negative, uric acid citrate not utilised, tolerate 1% sodium chloride but not 2% or more; acid production without gas from glucose, lactose, sucrose, fructose, xylose. Catalase positive, aerobic, optimum temperature 25–30°C. Thermal death point 60°C.

The bacterium isolated is closer to *Rhizobium* genus, than to any other bacterial genera<sup>2</sup>. Yet, the exact taxonomic position can be decided only after detailed studies of such symbionts.

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#### THE OCCURRENCE OF MUCOUS GLANDS IN THE APPENDAGES OF *TALORCHESTIA MARTENSII* (WEBER) AND *ORCHESTIA PLATENSIS* KRØYER (CRUSTACEA, AMPHIPODDA)

IN studies on the systematics and histophysiology of the amphipods *Talorchestia martensii* and *Orchestia platensis* it was observed that some of the appendages possess gland cells which stained characteristically with techniques like alcian blue, periodic acid-Schiff (PAS) (saliva fast) and safranin indicating the presence of mucoid material.

Although much work on the systematics of several families of amphipods has been done, no attention seems to have been bestowed till now on the occurrence of such glands in the appendages. It was felt necessary to study these glands.

*T. martensii* is a littoral form and occurs in abundance in damp places near Visakhapatnam harbour. *O. platensis*, a more terrestrial form, occurs in damp soil near banana vegetation far removed from the marine influence. Specimens were fixed in methanol-formaldehyde-acetic acid (MFA), alcoholic Bouin's, Susa or formol-calcium. After 24 hours of fixation they were washed and the appendages were pulled out carefully and stained according to the techniques given for mucopolysaccharides<sup>1-4</sup>. Bulk staining of whole specimens was also attempted but staining the individual appendages was found to be more convenient.

The gland cells are present in the antennule, antenna, the pereopods and uropods. None of the mouth parts, gnathopods and pleopods were found to possess them.

In the antennule they were present mostly in the first three segments of the peduncle. In the antenna the second and third joints possess them. In the different pereopods the distribution is slightly variable, the cells generally occur in almost all segments but are more numerous in the distal portions as in the pereopods 3 and 6. Their distribution in uropods appears more characteristic. Uropods 1 and 2 have more mucoid cells arranged in rows (Fig. 1) along the outer border of the segment. But the uropod 3 has only traces, in the ramus.

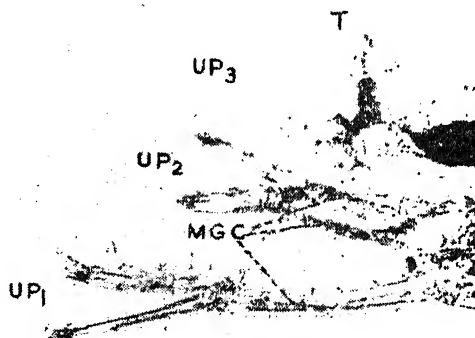


FIG. 1. Photograph of posterior region of *T. martensii* showing uropods with mucous glands (Alcian blue).

Up 1, Uropod 1; Up 2, Uropod 2; Up 3, Uropod 3; T, Telson; MGC, Mucous gland cells.

The histochemical tests carried out to detect the nature of these cells are given in Table I.

TABLE I  
Histochemical reactions

Test	Reaction	Remarks
Periodic acid-Schiff (PAS)	.. ++	
PAS (after saliva)	.. ++	
PAS (after pyridine extraction)	.. ++	
Aldehyde fuchsin	.. +	
Alcian blue	.. ++	
Bismarck brown	.. +	
Toluidine blue	.. Red	Gamnia metachromacy
Safranin O	.. Orange	Negative metachromacy
Sudan black B	.. -	

++ Intense reaction.  
+ Moderate reaction,  
- Negative reaction.

These tests allow us to conclude that the cells secrete mucopolysaccharides. Similar gland cells were observed in the region of the oesophagus around maxillae and maxillipedes. The precise functions of these gland cells in the appendages are well worth investigating further.

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# A NEW RECORD FOR PALAEMON (PALAEMON) CONCINNUS DANA, 1852 (DECAPODA, PALAEMONIDAE), FROM INDIA

THERE are relatively few studies on the smaller species of palaemonids in India. In view of their importance in the subsistence fisheries, we have undertaken an investigation of these and other prawns inhabiting the estuary and the system of

irrigation canals of river Krishna opening into the Bay of Bengal.

The present note is the first report of *Palaemon (Palaemon) concinnus* Dana, 1852, from the Indian subcontinent. One specimen each of this species was present in two samples collected in the irrigation canal off Nizampatnam. The relevant data are presented in Table I.

TABLE I  
*Palaemon concinnus* Dana. Salient measurements in the two specimens and rostral teeth formula (RTF)\*

Date	Sex	TL mm	CL mm	RL mm	RTF
July 1, 1973	♀	37	7	9	1+4+1 5
August 28, 1973	♂	40.5	7	10	1+5+1 5

\* In the rostral formula the three kinds of dorsal teeth: carapace, rostral and subterminal are indicated separately.

The specimens conform to the description of Holthuis<sup>1</sup>, but the salient characters are now stressed. In both specimens the rostrum (Fig. 1a) extends

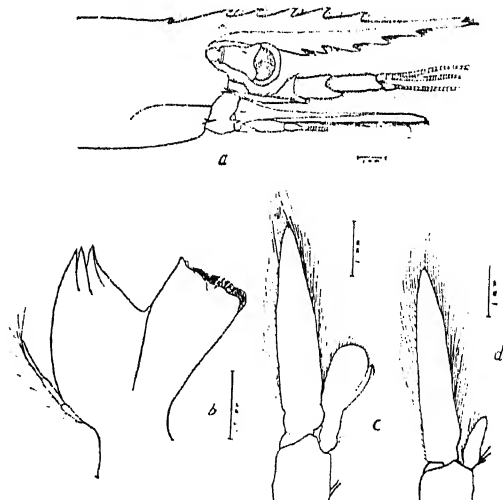


FIG. 1. *Palaemon (Palaemon) concinnus* Dana, 1852. a, anterior region of male; b, mandible; c, first pleopod of male; d, first pleopod of female.

distinctly beyond the scaphocerite. The first dorsal tooth is placed behind the orbital angle and there is a distinct dorsal subterminal tooth. According to Holthuis<sup>1</sup> the first and second dorsal teeth

TABLE II

Species	Locality	Collector and Year
<i>Palaemon debilis</i> Dana, 1852 (as <i>Leander gardeneri</i> )	Maldives	R. B. S. Sewell, 1923
<i>P. sewelli</i> (Kemp, 1915) (as <i>L. Sewelli</i> )	Goa; Ganjam coast	S. Kemp, 1916 'Investigator' 1890
<i>P. serrifer</i> (Stimpson, 1860) (as <i>L. serrifer</i> )	Bandra (in Greater Bombay)	J. W. Caunter, 1911
<i>P. pacificus</i> (Stimpson, 1860) (as <i>L. pacificus</i> )	Goa; Cape Comorin	S. Kemp, 1916 S. N. Pillay, 1911
<i>P. belindae</i> (Kemp, 1925) (as <i>L. belindae</i> )	Gulf of Mannar; Cape Comorin	S. Kemp, 1913 S. N. Pillay, 1911

articulate with "the rostrum proper", but his figure (Fig. 12a) does not indicate such articulation; our specimens conform to his figure. The fifth and sixth abdominal pleura end posteriorly in acute points, those of the sixth appearing almost spinous. The telson ends in a distinctly pointed tip.

The mandible bears a prominent palp which is distinctly three-jointed (Fig. 1b), as in the material examined by Holthuis<sup>1</sup>.

In the male, the carpus of the second pereopod is relatively longer than in the female. In the male, 1/3 of even the propodus of fifth pereopod extends beyond the scaphocerite, whereas in the female only the dactylus of this pereopod extends beyond it.

Typically, the endopod of the first pleopod in the male is provided mesially with a distinct rudimentary appendix interna which is absent in the female (Fig. 1c, d). The male bears a well-developed appendix masculina; the female is not sexually mature, because the pleopods are devoid of breeding setae.

Both specimens were captured in dragnets during low tide. The female occurred at a point midway between two regions where the salinity was 33.75‰ (at mouth) and 6.42‰ (higher up along canal), respectively. The male occurred at a point where the salinity was 4.02‰.

Holthuis<sup>1</sup> has shown that the species has been recorded from Suez in the west to the island of Makatea (in central Pacific) in the east, except for a wide gap between east Madagascar and Hongkong, from which region there is no record. According to Kemp<sup>2</sup>, five other species of the subgenus have been recorded from India (See Table II).

We have not come across any of the above five species in our collections, so far.

The author (K. R.) thanks the authorities of CSIR, New Delhi, for the award of a Research Fellowship.

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#### THE COMMON BAYA (*PLOCEUS PHILIPPINUS*) —A SERIOUS PEST OF AGRICULTURE

THE scientific assessment of the food of birds from the agricultural point of view was first made in this country by Maxwell-Lefroy in 1912. Ali (1936, 1972), Hassain and Bhalla (1937) and a few others gave some information on the food of birds affecting agriculture. Considering the importance of such studies and lack of sufficient data, feeding ecology and the mode of control of the common Baya, *Ploceus philippinus*, which is supposed to be a destructive pest of cereal crops of India was started early in 1968 in the West Bengal State Agricultural Farm, Chuchura. The farm encompasses an area of about 210 acres of which nearly 180 acres were cultivated and the remaining 30 acres were covered with bushes of *Lantana*, *Sesbania*, *Agave* and grasses. Apart from paddy and wheat, gram, corn, sugarcane and mustard were grown in the farm.

Birds were trapped in the farm between 7-30 A.M. and 9-30 A.M. After taking all necessary data like weight, temperature, etc., they were sacrificed for stomach analysis by usual procedure. The rate of digestion of rice grains was measured in two sets of experiments. Four Baya in each case was kept separately in cages through the night. The next morning when their stomach was empty, each of them was fed with 2 g of rice grain. At an interval of one hour, two hour, three and three and half hour, they were sacrificed. By opening the stomach the rate of digestion of rice grain was noted.

FOOD AND FEEDING ECOLOGY

The Common Baya is the most abundant bird species in the farm between April and September. Their number started declining from about the early January with the completion of harvesting. During their stay they prefer congregating among the Sisalhemp of plot 'D' and roosted there. When however, *Sesbania acutata* in the southern plot 'D' grew densely during the rainy season many of them shifted their roosting site to the branches of these plants.

An analysis of the stomach contents of 100 Baya reveals the percentage of occurrence of rice grains as 100, wheat 4, seeds of wild plants 5 and Coleoptera 1, Mollusca (Shell) 4, brick particles 4. The average weight of rice grain in each stomach is 2.2 g. Feeding experiment with captive Baya show that 2 g of rice grain has been digested by about 190 minutes. The average total hour of feeding of the Baya per day during July to January is about 10 hours. Therefore, each Baya consumes about 6 g of rice grain per day. So a single Baya destroys 180 g of rice per month or 5.400 kg per year. At Chuchura Agricultural Farm the Baya turns to other items of food in rare occasions. It appears therefore, that the staple diet of the Common Baya is paddy—thus a serious pest of crop.

However during the month of July bits of mulluscan shells are found in the stomachs of female Baya. Presumably the female Baya has fed on the shells to augment the supply of calcium which is necessary for the formation of their egg shell during the breeding season. It is also interesting to note that about 80% Baya studied have pieces of feathers in their stomach. No report to this inland birds is available. Pieces of feathers in the stomach of water birds, have, however, been reported by Wetmore (1920) which are believed to act as filter. But in land birds the significance of their presence could not be ascertained.

This work is being carried on by a grant from the American Museum of Natural History, New York.

The author is indebted to Dr. B. Biswas and Dr. Salim Ali for their advice and support through this study. Thanks are due to Dr. S. N. Banerjee, the then Additional Director of Agriculture, Government of West Bengal for permission to work in the Chuchura farm.

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ON THE PRESENCE OF A FORKED BARBEL  
IN A CAT FISH *CLARIAS BATRACHIUS*  
(LINNAEUS)

RECENTLY Tandon and Sharma (1971) claimed that the presence of a forked barbel in a fish is unknown in literature. Actually long before, Boulenger (1907) has reported a same case of forked barbel in the *Syndontis filamentosus*. Greenwood (1972) also observed the same phenomenon in some African Siluriform fishes, but has no published record.

Present specimen of *Clarias batrachus* (Linn.) Fig. 1 was caught from a lake of Bhopal on 9th September, 1972. It is a female with a total length of 25.5 cm and a weight of 148 gm (formaline preserved). The specimen has a forked right nasal barbel after 14 mm of its origin. The two arms of the fork are unequal. The forked barbel is smaller in total length than the left unforked barbel.

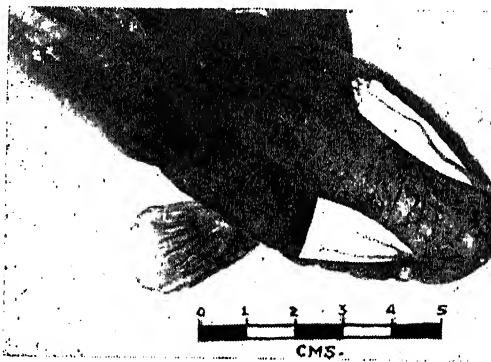


FIG. 1. On the presence of a forked barble in a cat fish.

According to Greenwood (1972) the abnormality may be congenital or the result of a regeneration, but in view of Tandon and Sharma (1971), it may be the result of some accidental injury as they were unable to produce a forked barbel in experimental regeneration of the barbels in another cat fish.



*Heteropneustes fossilis*: Still the significance of the phenomenon remains obscure.

I am indebted to Dr. P. H. Greenwood of British Museum (Natural History), London, for valuable information and going through the note. Thanks are also due to Prof. M. A. Qayyum for helpful suggestion and to Mr. Akhtar Hussain for the photograph.

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Bhopal (M.P.), December 24, 1973.

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#### RARE OCCURRENCE OF

#### *CONCHODERMA VIRGATUM* (SPENGLER, 1790) (CIRRIPIEDIA-LEPADOMORPHA) ON A SCHYPHOZOAN MEDUSA

*Conchoderma virgatum*, a pedunculate cirripede distributed world-wide, "is," according to available reports, found attached only to sub-surface objects. Its attachment to vertebrates, mostly fishes, has been recorded and reported by various investigators<sup>1-4</sup>. Its attachments to invertebrates, especially to copepod parasites, have also been reported<sup>5-6</sup>.

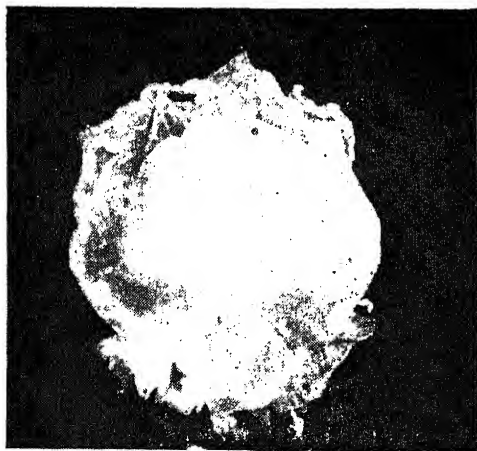


FIG. 1. Medusa with *Conchoderma virgatum* attached.

Presently, however, *C. virgatum* was found attached to *Rhopilema* sp. a shallow water schyphozoan medusa, collected at Tranquebar (11° 2' N, 79° 52' E) on 2nd September, 1973. The medusa measured 320 mm across and its

exumbrellar surface was uniformly covered with numerous small warts. A total of twenty-two barnacles were found attached to the medusa, twenty on the exumbrellar surface near the margin and the remaining two on the subumbrellar margin (Fig. 1). The morphological features of this barnacle agreed well with the description given by Annandale<sup>1</sup>. Both the medusa and the cirripedes remained alive for a day in the laboratory aquarium. Most of the specimens were of the same size measuring 14.5 mm in length and 7.0 mm in width.

This is the first time that *C. virgatum* was found attached to a medusa and is thus an interesting addition to the list of hosts for this barnacle.

Our thanks are due to Dr. R. Natarajan, Director, Centre of Advanced Study in Marine Biology, Porto Novo, for encouragement and facilities. One of us (A. S. F.) thanks the University Grants Commission, New Delhi, for the award of a Research Fellowship.

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#### *CALLOGOBIUS ANDAMANENSIS*. A NEW GOBIOID FISH FROM CURLLOW ISLAND. MIDDLE ANDAMANS. WITH A KEY TO SPECIES OF *CALLOGOBIUS* OF THE SEAS OF INDIA AND MALAY ARCHIPELAGO

IN 1970, during an ichthyological survey of the Andaman-Nicobar group of islands, the senior author collected eleven specimens of a fish belonging to the genus *Callogobius* ranging from 28.0–54.0 mm in standard length, from Curlow Island, Middle Andamans. These specimens differ from all the so far known species of *Callogobius* and are, therefore, described here as a new species and named as *Callogobius andamanensis*.

**Material.**—Eleven specimens including the holotype, 28.0–54.0 mm standard length, from Curlow Island, Middle Andaman, collected by A. G. K. Menon, on 24–1–1970.

**Measurements.**—Length of head, 25.0–34.4 (M = 29.2) per cent, height of body at D1 origin 14.0–16.7 (M = 16.5) per cent, at D2 origin 15.6–17.9 (M = 16.5) per cent of standard length,

Diameter of eye 18.1–25.0 ( $M=20.2$ ), bony interorbital space 13.6–20.8 ( $M=16.4$ ), snout 22.7–33.3 ( $M=28.9$ ) per cent of head length. First dorsal fin about as high as body, 14.4–20.0 ( $M=17.5$ ) per cent, second dorsal fin higher than the first, 17.9–25.0 ( $M=21.3$ ) per cent of standard length. First dorsal fin base 11.1–19.9 ( $M=14.9$ ) per cent, second dorsal fin base 28.2–35.7 ( $M=31.9$ ) per cent of standard length.

Distance between anterior tip of snout and origin of first dorsal fin 32.0–40.6 ( $M=36.8$ ) per cent, distance between anterior tip of snout and origin of second dorsal fin 45.7–59.3 ( $M=52.6$ ) per cent of standard length. Pelvic fin does not reach anal, shorter than head, 19.4–28.4 ( $M=23.0$ ) per cent, distance between anterior tip of snout and origin of pelvic fin 25.6–32.1 ( $M=28.3$ ) per cent of standard length. Pectoral fin slightly longer than head, 25.7–30.0 ( $M=28.0$ ) per cent, the distance between anterior tip of snout and origin of pectoral fin 27.7–32.1 ( $M=29.0$ ) per cent of standard length. Anal fin situated below second dorsal fin, length of the anal base 20.5–25.0 ( $M=22.7$ ), height 18.0–25.0 ( $M=21.9$ ), distance between the anterior tip of snout and the origin of anal 54.0–62.5 ( $M=58.9$ ) per cent of standard length. Caudal fin much longer than head, 33.3–42.0 ( $M=38.4$ ), length of caudal peduncle 20.0–25.0 ( $M=22.5$ ) per cent of standard length, width of caudal peduncle 36.0–50.0 ( $M=42.0$ ) per cent of caudal peduncle length. Anal papilla short, 2.3–5.1 ( $M=4.1$ ) per cent of standard length.

**Meristic characters.**—About 7 transverse papillated ridges and about 4–5 longitudinal papillated ridges (Figs. 1a–b). 10 short gill rakers. First dorsal fin with 6 rays, second dorsal with 10–13, pelvic 6, pectoral 14–18, anal 11–12, and caudal 14–17.

**Teeth.**—Several rows of pointed teeth in a band on both the lower and upper jaws (Fig. 1c), teeth near symphysis slightly longer than the rest, outer rows of teeth of the lower jaw curved inwards.

**Scales.**—Cycloid throughout (Fig. 1e), very minute, slightly larger posteriorly, about 70 rows along longitudinal series, 11–14 in transverse series counted from origin of anal fin, about 30 in predorsal series; from 2nd dorsal anteriorly the scales are much smaller and covered by a thin granulous skin and not clearly visible externally; embedded scales on cheek and opercle; breast naked.

**Colouration.**—Brownish, mottled with 2–3 indistinct darker blotches; dorsal and anal fins dusky, caudal and pectoral fins with dark bars; breast and abdomen dull brown.

**Distribution.**—Curlow Island, Middle Andaman. **Holotype.**—In Zoological Survey of India, Calcutta, Reg. No. F. 7105/2; Curlow Island, Middle Andaman, 54.0 mm S.L., collected by A. G. K. Menon, on 24–1–1970.

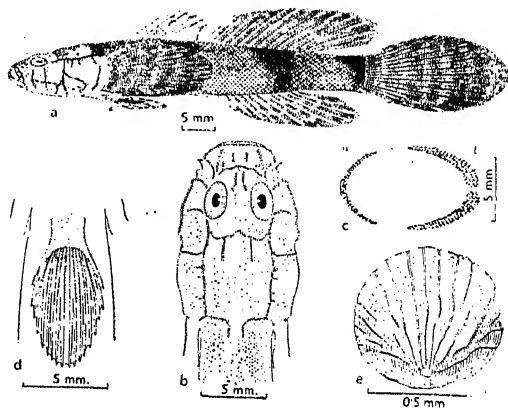


FIG. 1. *Callogobius andamanensis* Menon and Chatterjee. a, Lateral view of Holotype (Z.S.I. Reg. No. F. 7105/2). b, Dorsal view of the head of the same. c, Teeth of the same. d, Pelvic fin of the same. e, Scale of the same.

**Paratypes.**—Ten in Zoological Survey of India, Calcutta, Reg. No. F. 7106/2; 28.0–51.0 mm S.L., bearing the same data as holotype.

**Diagnosis and Affinities.**—*Callogobius andamanensis* is closely related to *C. liolepis* (Bleeker) Koumans, and *C. mannarensis* Rangarajan in having cycloid scales throughout the body, but the new species can be easily distinguished from the former by the larger number of longitudinal series of scales (70 versus 45), and from the latter by the nature of the arrangement of teeth, teeth being arranged in several rows in *C. andamanensis* whereas they are biserial in *C. mannarensis*.

KEY TO THE IDENTIFICATION OF THE SPECIES OF  
*Callogobius* OF THE SEAS OF INDIA AND  
MALAY ARCHIPELAGO

1. Scales ctenoid posteriorly, cycloid anteriorly . . . 2
2. 40–45 series of scales . . . *C. hassettii* (Blkr.)
- 28–32 series of scales . . . 3
3. Basal membrane of Pelvic fin absent . . . *C. sclateri* (Stndur.)
- Basal membrane of Pelvic fin present . . . 4
4. 11–13 rays in 2nd dorsal . . . *C. seshaiyai* (Jacob and Rangarajan)
- 10 or less rays in 2nd dorsal . . . 5

5. 10-12 Predorsal scales ..	<i>C. centrolepis</i> Weber
8 Predorsal scales ..	<i>C. snelli</i> Kumans
6. Predorsal scales absent, teeth biserial ..	<i>C. mannarensis</i> Rangarajan
Predorsal scales present, teeth in several rows ..	7
7. $\pm 45$ series of scales ..	<i>C. liolepis</i> (Blkr.) Koumans
$\pm 70$ series of scales ..	<i>C. andamanensis</i> Menon and Chatterjee

Division of Fishes, A. G. K. MENON.  
Zoological Survey of India, T. K. CHATTERJEE.  
Calcutta, December 14, 1973.

### OCCURRENCE OF STYLAR OBTURATOR IN TWO EUPHORBIACEAE

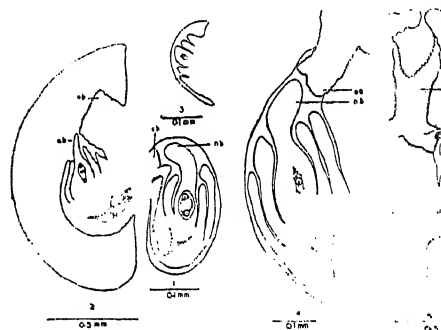
IN the various floras and systematic works, the ovule in the Euphorbiaceae is described as anatropous and pendulous with a ventral raphe and the micropyle turned upwards. Willis<sup>1</sup> mentioned that the ovules are constant throughout the family and form one of its distinctive features. Cronquist<sup>2</sup> used the term epitropous implying that the ovule is anatropous with the micropyle turned upwards. Davis<sup>3</sup> and Rao<sup>4</sup> mentioned that besides the anatropous ovules, orthotropous and hemianatropous ovules also occur in the family. Webster<sup>5</sup> holds that the ovules are 'prevalingly anatropous, but hemianatropous ovules are common in the tribe Phyllanthae. Irrespective of the type of the ovule, the obturator was, however, found to be of placental origin in all the taxa of the Euphorbiaceae investigated so far.

A careful and comparative study of the ovule and obturator in 21 taxa of the Euphorbiaceae, listed below tribewise, has been made by us and observations recorded here:

Tribe	Species investigated
Phyllanthae	<i>Breynia cernua</i> Muell. Arg.
	<i>B. rhamnoides</i> Muell. Arg.
	<i>Chorisandra pinnata</i> Wt.
	<i>Meineckia parvifolia</i> (Wt.) Webster
	<i>Kirganelia reticulata</i> Baill.
	<i>Phyllanthus acidus</i> (Linn.) Skeels.
	<i>P. debilis</i> Herb. Ham.
	<i>P. odontodeni</i> Muell. Arg.
	<i>P. rotundifolius</i> Klein.
	<i>Sauropus quadrangularis</i> Muell. Arg.
	<i>Synostemon bacciforme</i> (Linn.) Webster.

Tribe	Species investigated
Brideliaceae	<i>Cleistanthus collinus</i> Benth.
	<i>Bridelia montana</i> Willd.
	<i>B. retusa</i> Spreng.
	<i>B. stipularis</i> Blume.
Acalyphaceae	<i>Macaranga peltata</i> Muell. A.
	<i>Mallotus roxburghianus</i> Muell. A.
	<i>Trewia nudiflora</i> Linn.
Jatrophaeae	<i>Jatropha glandulifera</i> Roxb.
	<i>J. heterophylla</i> Heyne.
Euphorbia	<i>Euphorbia perbracteata</i> Gage

It seems that the whole axis is placental and that the ovules may arise at from the apex to the base but the n always faces upwards. In the members tribes Brideliaceae, Acalyphaceae, Jatrophaeae, biaeae, *Meineckia parvifolia* and *Kirganelia* of the Phyllanthae, the ovules are attached upper part of the axis and are typically an and pendulous (Fig. 1). In these, the



Figs.1-5. Fig. 1. L.S. ovule in *Meineckia parvifolia* showing anatropous ovule and obturator. Fig. 2. L.S. ovule in *Chorisandra pinnata* showing hemianatropous ovule and obturator. Fig. 3. L.S. ovule of *Breynia cernua* at the mmc stage (The mmc becomes nucellar later). Fig. 4. L.S. ovule of *B. cernua* at the Megaspore Tetrad stage. Fig. 5. L.S. ovary in *B. rhamnoides* showing stylar nucellar beak, ob-obturator, st-Style (transmitting tissue).

was found to be of placental origin as Euphorbiaceae. In *Chorisandra pinnata*, *Phyllanthus*, *Sauropus quadrangularis* and *Stemon bacciforme*, the ovules are attached the middle part of the axis and are hemianatropous (Fig. 2). In these also, the obturator is of placental origin.

But, *Breynia rhamnoides* and *B. cernua* are from the above, in that the ovules are at the base of the axis and are curved towards the apical part of the ovary (Figs. 4, 5). TI

remains very short and the body of the ovule turns upwards through  $90^\circ$  from its region of attachment, consequently bringing the apical part of the ovule to the base of the style (Figs. 3-5). Thathachar<sup>6</sup> described the ovules in *Breynia* as orthotropous. But the ovules in *Breynia* do not conform to the definition of an orthotropous condition but are nearer to the hemianatropous nature. The obturator in these two species is clearly stylar in origin, as the glandular cells of the stylar canal descend in a bundle and come in touch with nucellar beak which protrudes out of the micropyle (Figs. 4, 5). This note records this feature for the first time to the best of our knowledge. The cells of the obturator, transmitting tissue, and the stigmatic papillae are remarkably similar and form a continuous strand of tissue through the style. Earlier workers on *Breynia*<sup>6,7</sup>, somehow missed to make out the stylar origin of the obturator in *Breynia*.

One of us (D. S. R.) is thankful to the C.S.I.R., for the award of a Junior Research Fellowship.

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## SHORT SCIENTIFIC NOTES

### A Note on Pyrrhotite of Tapang, Orissa

The note embodies preliminary investigations on pyrrhotite and associated sulphide mineralisation around Tapang ( $20^\circ 5' 35''$  N;  $85^\circ 35' 30''$  E), Orissa. The rocks of the area consist of charnockites, basic granulites, granitic rocks, khondalites and quartzites. Pyrrhotite is invariably confined to the migmatized parts of basic granulites only. The sulphides occur along joints and fractures, occasionally as massive but otherwise sporadically dispersed.

Pyrrhotite is the dominant sulphide constituting more than 90% of the ore body. Coarse aggregates of anhedral pyrrhotite grains are interspersed with finer grained clusters with inclusions of silicate minerals. Etch reactions with  $\text{HNO}_3$  (1:1) and  $\text{HCl}$  (1:1) show strong brown colour and feeble tarnish respectively. With  $\text{FeCl}_3$  and  $\text{HgCl}_2$  negative reactions were recorded. Besides pyrrhotite, a few other sulphides like chalcopyrite, pentlandite, sphalerite, marcasite and pyrite occur in very minor quantities.

Chemical analyses of four samples of pyrrhotite showed the presence of nickel from 0.11% to 1.17% and sulphur from 33.4% to 37.7%. Besides, very small amounts of copper and zinc were also detected.

In the present case, pyrrhotite might be considered as having been formed at the time of crystallisation

of basic rocks but selective occurrences of the sulphides along zones of migmatization definitely indicate a genetic relationship with granitic activity. Therefore the alternative possibility of sedimentogenous origin or an origin by inversion from pyrite can be ruled out. Observed textural features and distribution pattern of the sulphides in basic granulites support the above contentions. Therefore it is logical to associate pyrrhotite with migmatization of basic granulites. The granitic material, while moving up along foliation planes, could have carried the sulphides and localised them at suitable sites under favourable conditions. This explains why pyrrhotite is observed wherever migmatitic activities are prominent.

Department of Geology,  
Ravenshaw College,  
Cuttack-3, September 17, 1973.

K. N. SAHU.

### A Note on Some Interesting Fungi from Hyderabad (India)

During the survey (1971-72) of microfungi from pond waters, pond muds and soils of Hyderabad District (A.P., India) the author has isolated 146 species; which include 43 Phycomycetes, 16 Ascomycetes, 87 Fungi Imperfecti and a single sterile

TABLE I  
Distribution of some interesting Fungi in pond waters, pond muds and soils

Fungus	Pond waters pH range 5.8-8.2	Pond muds pH range 7.0-8.6	Soil pH range 6.8-9
* <i>Achlya debaryana</i> Humphrey	..	+	+
* <i>A. klebisiana</i> Pieters	..	+	+
* <i>A. orion</i> Coker and Couch	..	+	+
* <i>A. proliferoides</i> Coker	..	+	+
* <i>A. recurva</i> Cornu	..	+	+
* <i>Aphanomyces</i> sp.	..	+	+
** <i>Dictyosporium sacchari</i> (Stevenson) Damon	..	..	..
* <i>Neurospora crassa</i> Shear and Dodge (Conidial stage)	..	..	..
* <i>Penicillium brefeldianum</i> Dodge	..	..	..
* <i>P. rubrum</i> Stoll.	..	..	+
* <i>P. turbatum</i> Westling	..	..	+
* <i>Periconia saraswatipurensis</i> Bilgrami	..	..	+
** <i>Stachybotrys cylindrospora</i> Jensen	..	..	+
† <i>Thielaviopsis parasoxa</i> (Dade) Moreau	..	..	..
* New records for South India.	** New additions to Indian soils.		
† New addition to mud microfungi.	† Present                      Absent.		

mycelial isolate. Table I shows the distribution of some interesting fungi in pond waters, muds and soils.

*Dictyosporium sacchari* and *Stachybotrys cylindrospora* which form new records for Indian soil are described below.

*Dictyosporium sacchari* (Stevenson) Damon in *Bull. Torrey Bot. Club*, 1953, 80, 164.

Colonies on potato sucrose agar medium black, compact, effuse; hyphae 3.2-5.0  $\mu$  wide; conidiophore mother cells 4.0-6.4  $\mu$  wide, 3.0-4.8  $\mu$  long; conidiophores are upto 150  $\mu$  long, 4-6  $\mu$  thick, straight, cylindrical, colourless to pale brown except for the thick or dark brown septa, smooth to minutely verruculose; conidia square, spherical or sub-spherical, flattened in one plane, cruciately septate, 4-celled, mid to dark brown; verruculose, 9.0-17.6  $\mu$  in diameter, in face view 9-14  $\mu$  thick.

Isolated from forest soil, Vikarabad, October, 31, 1971, pH 7.3 (OUF 33; IMI 161794).

*Stachybotrys cylindrospora* Jensen, in *Bull. Univ. Agric. Exp. Sta.*, 1912, 315, 496.

Colonies growing well on potato sucrose medium, black, mycelium superficial and in hyphae forming ropes, 2.0-4.0  $\mu$  wide; conidiophores with hyaline lower part, the upper part verruculose or smooth, 48-65  $\mu$  long, 2.0-3.5  $\mu$  wide at the base; phialides 8-11  $\mu$  long, 6.0  $\mu$  wide at the base; conidia cylindrical, rounded at the base, rounded or truncate at the base, grey with dark longitudinal striations, 5-10  $\mu$  long, 1.6-3.6  $\mu$  wide.

Isolated from forest soil and pond water, Vikarabad, April 16, 1972, pH 7.3 (OUF 33).

The author expresses his thanks to: Dr. D. Ellis, CMI, Kew, England, for help in the identification, to Dr. P. Ramarao, Reader, Botany Department, for guidance and to Prof. M. R. Dean, Faculty of Science, Osmania University, for kind encouragement.

Mycology and Plant Pathology Lab., C. MANOHARA

Department of Botany, Osmania University, Hyderabad-500007 (A.P.), India, December 22, 1973.

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# The First Record of Algal Bioherms in the Palaeozoic Sequence of the Tethyan Zone of the Kumaun Himalaya

A fairly thick Palaeo-Mesozoic sequence has been known to occur in the Kumaun Himalaya for a long time and has been studied by a number of workers<sup>1-5</sup>. In a recent visit to the area as members of an expedition sponsored by the Wadia Institute of Himalayan Geology to the Pailkhand region of the Chamoli District (U.P.) the present authors located for the first time a 45 metre thick band of biohermal limestone, which is full of interest and this note is intended to record the same.

The Palaeozoic sequence with the thickness of individual units along the Yong valley, where this limestone is exposed, is as follows :

	Thickness
Kuling Shale	20 m
unconformity	-----
Muth Quartzite	150 m
Variegated Series	50 m
Biohermal limestone	45 m
Shiala Series	500 m
Garbyang Series	1000 m (approximately)

The Shiala Series containing typical Ordovician fossils comprises bands of fine-grained pale to olive green shales alternating with biostromal limestone. The latter bears characteristic algal structures, crinoidal stems and distorted and compressed shells. These biostromal bands range in thickness from a few centimetres to about a metre each. Apart from these bands, the limestone also occurs as lensoid bodies within the dominantly shaly formation. Towards the upper part of this

formation the biostromal limestone increases in extent and thickness with concomitant subordination of the shale.

Immediately overlying the Shiala Series with a perfect conformity a green biohermal limestone is exposed near the deserted village of Yong, which comprises essentially branching algal material within which deformed corals, stromatopores, bryozoan and crinoidal stems are found. This limestone exhibits a mushroom like domal structure which is about 750 metres across and has 45 metres of exposed thickness at the core. The core is almost entirely composed of the organic material whereas towards the margins there are intervening layers of terrigenous sediment and imperceptibly it merges into green biostromal limestone. A chain of such reefal structures is exposed throughout the Yong valley upto Rinkhim and these can be easily located even from a distance due to their characteristic helmet-like disposition. The Variegated Series overlies the reefs with a discordance although it is concordant with the intervening biostromal limestone. This would indicate that the reefs started growing on a more or less even sea floor and thereupon quickly outgrew the surrounding sedimentation, the reef detritus possibly contributing necessary material for the biostromal limestone around the bioherms. The succeeding Variegated Series seems to have been deposited on an uneven surface produced by the reefal growths.

The occurrence of algal bioherms in the Palaeozoic sequence of Tethyan zone has an important palaeogeographical significance, for generally algal bioherms and biostromes are typical of bank environment<sup>6</sup> and a definite shallowing of Tethyan sea towards the end of Ordovician and early Silurian is indicated.

The authors are grateful to Prof. A. G. Jhingran, Hon. Director, Wadia Institute of Himalayan Geology, for organizing the Expedition and encouragement. Thanks are also due to Dr. K. S. Valdiya for useful discussions.

Wadia Institute of Himalayan Geology,  
Delhi-110007, December 14, 1973.

S. K. SHAIL  
A. K. SINHA.

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## REVIEWS AND NOTICES OF BOOKS

**Annual Review of Astronomy and Astrophysics** (Vol. 11). Edited by L. Goldberg, D. Layzer and J. G. Phillips. (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306), 1973. Pp. vii + 435. Price : U.S.A. \$ 12.00 ; elsewhere : \$ 12.50.

Perhaps more than most other areas of science, the fields of astronomy and astrophysics are expanding at a staggering rate, both from the point of view of new knowledge being acquired, and new work being published. Just ten years ago, for example, it was possible for the average radio astronomer to have a reasonably complete grasp of his field—instrumentation, observation, and theory; today this is clearly beyond the realm of possibility. It is for this reason that systematic reviews are becoming increasingly popular, indeed essential.

The series *Annual Review of Astronomy and Astrophysics* is an indispensable contribution in this regard. Started in 1962, its editorial committees have always included some of the top names in these fields, and the authors themselves are invariably distinguished representatives of their specialities. The series is well-integrated, in the sense that each volume contains cumulative author and subject indexes covering earlier volumes, and there are cross-references to related articles appearing in other *Annual Reviews*.

This eleventh volume contains 15 review articles, and as usual these cover a wide spectrum. Three articles deal with instrumentation—image tubes, new optical telescope systems, and the fast-growing field of mm-wavelength radio astronomy. Four articles are concerned with stars—spectral classification, explosive nucleosynthesis, carbon stars, and non-LTE conditions in stellar atmospheres. Four others

cover recent work on the interplanetary and stellar medium—interplanetary scintillations, lapsing interstellar clouds, interstellar grains, turbulence in the interstellar plasma. Then there are two papers dealing with cosmology, one on system kinematics, and one on diffuse X and  $\gamma$  radiation. The articles generally cover work of the past decade, up to and including 1972.

P. SHANKAR

### Award of Research Degrees

M.S. University of Baroda has awarded Ph.D degree in Physics to Kumari Manoj Chakravorty; Ph.D. degree in Geology to Devendra Pal.

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Zoology to Shri V. Devanarayan Naidu; Ph.D. degree in Geology to Shri G. Lakshmi Reddy.

### Books Received

*Annual Review of Microbiology* (Vol. 27). (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306, U.S.A.), 1973. Pp. xx + 650. Price \$ 12.00 ; elsewhere \$ 12.50.

*Annual Review of Plant Physiology* (Vol. 24). (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306, U.S.A.), 1973. Pp. viii + 650. Price \$ 12.00 ; elsewhere \$ 12.50.

*Annual Review of Phytopathology* (Vol. 11). (Annual Reviews, Inc., 4139, El Camino Way, Palo Alto, California 94306), 1973. Pp. vi + 559. Price \$ 12.00 ; elsewhere \$ 12.50.

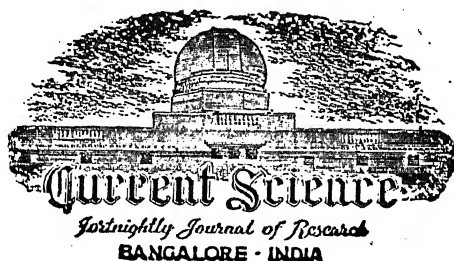
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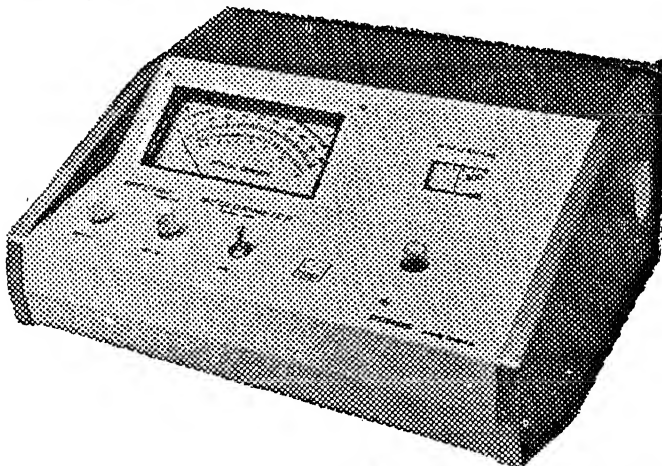
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# RELATION BETWEEN THE $n^{\text{th}}$ POWER OF THE ADJACENCY MATRIX AND THE NUMBER OF POINTS OF A COMPLETE GRAPH

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## ABSTRACT

In this note a theorem is proved by induction, which expresses the  $n^{\text{th}}$  power of the adjacency matrix of a complete graph in the form of sum of a series of  $n \times n$  matrices. The elements of these matrices being functions of  $p$ , the number of vertices of the complete graph  $K_p$ . This relation is useful in finding the number of walks of length  $n$  from any point of the graph to the other.

**DEFINITIONS:** A graph  $G$  consists of a finite non-empty set  $V$  of  $P$  points, together with a set  $X$  of unordered pairs of distinct points of  $V$ . The points of  $V$  are called vertices and the elements of  $X$  are called edges. Each pair  $x = u, v$  of points in  $X$  is a line of the graph  $G$  and  $x$  is said to join  $u$  and  $v$ . We write  $x = uv$  and say that  $u$  is adjacent to  $v$ .

A graph  $G$  is said to be complete, if the edge set  $X$  consists of all possible pairs of distinct points of  $V$ , and is denoted by  $K_p$ .

A graph is said to be labelled, if each of its vertices are designated by a name, viz.,  $V_1, V_2, \dots, V_p$ . A walk in a graph is an alternating sequence of vertices and edges, which are incident with each other. The length of a walk is the number of edges that it contains.

The Adjacency matrix of a graph with  $P$  vertices is defined as a Square matrix of order  $P^2$ , denoted by  $A$  whose elements are such that

$$a_{ij} = \begin{cases} 1 & \text{if } v_i \text{ is adjacent to } v_j \\ 0 & \text{otherwise} \end{cases}$$

The purpose of this note is to establish an identity relating the Adjacency matrix and the number of vertices  $P$  of a complete graph.

**Theorem.**—The adjacency matrix of a complete graph  $K_p$  with  $P (\geq 3)$  vertices satisfies the following Identity for all positive integral values of  $n$ .

When  $n$  is odd we have,

$$[A]^n_{p \times p} = I_{p \times p} + \sum_{r=0}^{(n-3)/2} [(p-1)^{2r+1}(p-2)]_{p \times p}$$

When  $n$  is even, we have

$$[A]^n_{p \times p} = [A]_{p \times p} + \sum_{r=0}^{(n-2)/2} [(p-1)^{2r}(p-2)]_{p \times p}$$

Here  $I_{p \times p}$  is the Identity matrix and  $[(\quad)]_{p \times p}$  represents a square matrix of order  $p^2$ , the elements of which are in the brackets.

**Proof:** The proof is by Induction on  $n$ .

$$\text{for } n=1 \quad A = A$$

$$\text{for } n=2 \quad [A]^2 = I_{p \times p} + [(p-2)]$$

$$\text{for } n=3 \quad [A]^3 = [A] + [(p-1)(p-2)]$$

$$\text{for } n=4 \quad [A]^4 = I_{p \times p} + [(p-2)] + [(p-2)(p-1)^2].$$

These four cases can be verified by actual multiplication of the matrix  $(A)$  we have,

$$A = a_{ij};$$

$$a_{ij} = \begin{cases} 0 & i=j \\ 1 & i \neq j \end{cases}$$

$$A^2 = a_{ij}^{(2)}$$

$$a_{ij}^{(2)} = \begin{cases} (p-1) & i=j \\ (p-2) & i \neq j \end{cases}$$

$$A^3 = a_{ij}^{(3)};$$

$$a_{ij}^{(3)} = \begin{cases} (p-1)(p-2) & i=j \\ (p-1) + (p-2)^2 & i \neq j \end{cases}$$

$$A^4 = a_{ij}^{(4)};$$

$$a_{ij}^{(4)} = \begin{cases} (p-1)(p-1) + (p-2)^2 & i=j \\ (p-2)2(p-1) + (p-2)^2 & i \neq j \end{cases}$$

**Case 1.**—The identity was seen to be true for 2 and 4. Let us assume that the Identity is true for some even number  $K$  then for  $K+1$ , an odd number we must obtain the other form of the Identity. Therefore, for  $n+k$  even we have,

$$[A]^k = I_{p \times p} + \sum_{r=0}^{(k-2)/2} [(p-1)^{2r}(p-2)]_{p \times p}.$$

Now

$$[A]^{k+1} = [A][A]^k$$

$$[A]^{k+1} = [A]I + \sum_{r=0}^{(k-2)/2} [A][(p-1)^{2r}(p-2)]$$

We have

$$[A][(p-1)^{2r}(p-2)] = [(p-1)^{2r+1}(p-2)]$$

$$[A]^{k+1} = [A] + \sum_{r=0}^{(k-2)/2} [(p-1)^{2r+1}(p-2)]$$

Put  $k+1 = m$  some odd integer, then

$k = m-1$ , Hence we have

$$[A]^m = [A] + \sum_{r=0}^{(m-3)/2} [(p-1)^{2r+1}(p-2)],$$

Case 2.—The Identity was seen to be true for 1 and 3. Let the Identity be true for some odd integer  $K^1$ , then  $K^1+1$  will be an even integer. now we must obtain the other form of Identity which is true for even integer.

We have for  $n = K^1$  an odd number

$$[A]^{k^1} = [A] + \sum_{r=0}^{(n-1)/2} [(p-1)^{2r+1}(p-2)]_{p,p}$$

Now

$$[A]^{k^1+1} = [A] [A]^{k^1}$$

$$[A]^{k^1+1} = [A] [A] + \sum_{r=0}^{(n-3)/2} [A] [(p-1)^{2r+1} \times (p-2)]_{p,p}$$

$$[A]^2 = [I] + [(p-1)^0(p-2)]$$

$$[A] [(p-1)^{2r+1}(p-2)] = [(p-1)^{2r+2}(p-2)]$$

$$[A]^{k^1+1} = I_{p,p} + [(p-1)^0(p-2)] + \sum_{r=0}^{(k-3)/2} [(p-1)^{2r+2}(p-2)]_{p,p}$$

$K^1+1$  is an even number say  $n$ .

then

$$[A]^n = I_{p,p} + \sum_{r=0}^{((K^1-1)/2)+1} [(p-1)^{2r}(p-2)]$$

$$[A]^n = I_{p,p} + \sum_{r=0}^{(n-2)/2} [(p-1)^{2r}(p-2)]_{p,p}$$

Hence the proof :

The Identity can also be proved for even number and odd numbers separately by considering the respective cases for numbers like  $n$  and  $n+2$ . It may be noted that the above proved Identity helps one to calculate the  $(ij)$  element  $a_j^{(i)}$  of  $A^n$ , which represents the number of walks of length  $n$  from  $i$  to  $j$ .

#### ACKNOWLEDGEMENT

The author acknowledges the guidance of Dr. E. Sampath Kumar, Karnatak University in preparing this note.

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### THE PHYSIOLOGICAL SIGNIFICANCE OF THE INTERACTION OF BILIRUBIN WITH RECONSTITUTED COLLAGEN FIBRILS\*

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THE extensive yellowing of the body surface in hyperbilirubinaemic new borns and the restoration to the normal colour of the skin after recovery from jaundice suggest that skin takes active part in the homeostasis of bilirubin at least during the diseased condition. Evidence has been adduced earlier to show that skin epithelium and skin strips of the mouse, rat, guinea pig and man possess a mechanism to accumulate and release bilirubin<sup>1,2</sup>. When skin strips saturated with bilirubin are exposed to light (80 watt) in Krebs Ringer buffer, there is a rapid bleaching of the skin accompanied by the release of water soluble and non-diazotizable degradation products of bilirubin<sup>3</sup>.

The uptake of bilirubin by skin is sensitive to temperature indicating that binding of bilirubin to skin strips involves participation of collagen. Results of studies on the binding of bilirubin to collagen fibrils are reported now which suggest

that the interaction between collagen and bilirubin may be involved in the uptake of bilirubin by skin.

Collagen was prepared from rat tail tendons according to Glimcher and Krane<sup>4</sup> and purified by dialysis against 0.02 M  $\text{Na}_2\text{HPO}_4$ . The precipitate obtained was redissolved in 1% acetic acid and stored as a lyophilized material. It contained 13.5% hydroxyproline<sup>5</sup>. When required it was dissolved in 100 mM acetic acid and converted to the reconstituted fibrillar form by dialysing at 2° against 200 mM Tris HCl buffer pH 8.6 or 7.5 as required following essentially the procedure of Gross and Krick<sup>6</sup>. The interaction of bilirubin with collagen fibrillar aggregate was followed in 5 ml 100 mM Tris HCl buffer pH 8.6 or 7.5 at 37° C in a two phase system where collagen was present as an opaque rigid gel composed of striated fibrils and bilirubin was in aqueous solution. Different amounts of serum albumin or competing anions could be added to this system as desired. The collagen fibrils were recovered by centrifugation and washed repeatedly with 100 mM Tris HCl buffer and the washed fibrils extracted

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with a solvent system made up of acetone : ethanol : water : acetic acid (v/v, 7:7:4:2) and bilirubin estimated in the extract according to Van Roy *et al.*<sup>7</sup>

As shown in Fig. 1 the interaction of bilirubin with collagen fibrils was affected by concentration of bilirubin in the incubation medium. Binding of bilirubin to collagen fibrils was found to be a linear function of its concentration upto 340  $\mu$ M reaching saturation at 425  $\mu$ M of bilirubin and the binding was complete within 60 min. At temperatures below 5° C, binding was depressed and uptake was altogether abolished by heat denaturation of collagen fibrils. The amount of bilirubin bound with unheated control collagen fibrils was 380  $n$  mol/mg collagen fibrils whereas after heat denaturation at 52–53° C for 1 hr collagen fibrils showed negligible binding affinity for bilirubin.

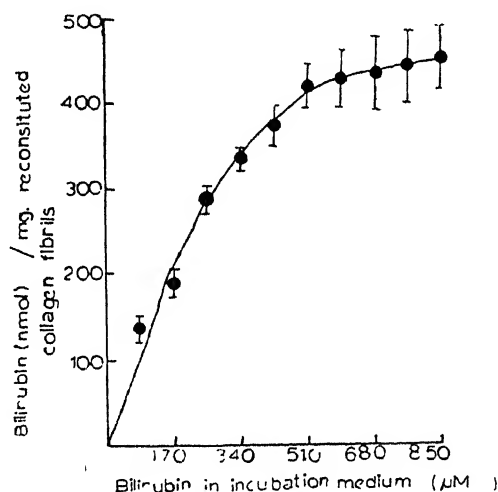


FIG. 1. Binding of bilirubin to collagen fibrils as affected by concentration of bilirubin. Collagen fibrils (0.5 mg) were incubated in 5 ml 100 mM Tris HCl buffer (pH 8.6) containing increasing 85–850  $\mu$ M concentration of bilirubin at 37° C for 1 h. The binding of bilirubin with collagen fibrils was estimated as described in text. The vertical bars represent the S.D. of the mean value from three separate experiments.

By forming a characteristic bilirubin : albumin complex serum albumin is known to reverse the binding of bilirubin to lipids. The binding affinity of collagen fibrils for bilirubin was, therefore, investigated in different molar concentrations of human serum albumin. Human serum albumin in a concentration of 40–80  $\mu$ M dissociated only 50% of bilirubin from collagen fibrils : bilirubin complex as shown in Table I.

Salicylate or sulfonamide used in the treatment of neonates does not affect the binding of bilirubin to collagen fibrils. These drugs readily dissociate

TABLE I

*Effect of human serum albumin on the dissociation of bilirubin from bilirubin : collagen fibril complex*

Bilirubin : collagen fibrils complex were prepared by incubating collagen fibril (0.5 mg), in 5 ml 100 mM Tris HCl buffer (pH 8.6) containing 425  $\mu$ M bilirubin at 37° C for 1 h, washed free from medium as described in the text. The fibrils were then incubated, in 5 ml Tris HCl buffer (pH 8.6) in presence of 40–80  $\mu$ M of human serum albumin (HSA). The dissociation of bilirubin was followed for 4 h. Each result is the mean of the values from two separate experiments. Initial bilirubin content was 360  $n$  mol/mg collagen fibrils.

Time (h)	Bilirubin ( $n$ mol) dissociated/mg of collagen fibrils in presence of	
	40 $\mu$ M HSA	80 $\mu$ M HSA
0	~	~
2	144	150
4	162	180

the bilirubin from serum albumin : bilirubin complex<sup>9</sup> and increases the incidence of kernicterus<sup>10</sup>.

Urea (8.5 M) is known to alter the cross-linking property of collagen fibrils. It was of interest, therefore, to investigate the effect of urea in the binding of bilirubin to collagen fibrils. The results presented in Table II show that urea at 4 M concentrations in the incubation medium diminished 70% of the binding of bilirubin to collagen fibrils.

TABLE II

*Effect of urea on the binding of bilirubin with reconstituted collagen fibrils*

Collagen fibrils (0.5 mg) were incubated in 5 ml 100 mM Tris HCl buffer (pH 8.6) containing 425  $\mu$ M bilirubin and increasing concentration of urea at 37° C for 1 h in a metabolic shaker and binding of bilirubin with collagen fibrils was estimated as described in the text. Each result is the mean of the value from two separate experiments.

Urea concentration (M)	Bilirubin ( $n$ mol) / mg collagen fibrils
None	386
1	256
2	192
3	146
4	102

Urea denaturation and thermal denaturation studies throw light on the importance and organization of the helical structure of collagen fibrils on its affinity for bilirubin. These observations may be of physiological significance in view of the fact that skin provides a semi-solid matrix for the connective tissue. The importance of the role of collagen fibrils is further strengthened by the observation on the lack of affinity of gelatin for bilirubin under identical conditions.

The liver conjugate being still in a developing state may not be able to cope up with all the bilirubin encountered in neonatal jaundice. In such a situation it is presumable that the skin takes the main load of free bilirubin employing native collagen as the binding agent. Collagen thus provides a matrix for the photooxidation of bilirubin to relatively polar degradation products which are then eliminated by the kidney.

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#### ECOLOGICAL IMPLICATIONS OF HAEMOLYMPH PROTEIN PATTERNS IN SOME AMPHIPOD AND ISOPOD SPECIES

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**A**MONG the numerous proteins that are to be found in the crustacean haemolymph, two particular components have been easily recognized, when present, owing to their obvious properties—the respiratory pigments and the coagulable proteins. The chemical nature and physical properties of both these proteins have been recently reviewed by Jenuiaux<sup>6</sup>, Redmond<sup>9</sup> and by Grégoire<sup>5</sup>.

The remaining protein fraction has been resolved into a series of components with different electrophoretic properties<sup>1,2,3,7,11,12</sup>. Although the nature of these proteins is poorly understood, their electrophoretic mobilities in some cases have been used tentatively in taxonomic studies<sup>7</sup>.

The aim of the present investigation is to define some of the characteristics of haemocyanin and other blood proteins in a few marine, freshwater and terrestrial isopods, and to determine if the haemolymph protein electrophoretic pattern in these

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species has any taxonomic or ecological implications.

#### MATERIAL AND METHODS

##### Animals

##### AMPHIPODA :

1. *Gammarus fossarum* Koch : ♀♀ 8–11 mm, ♂♂ 10–14 mm, collected from a stream originating from the Niebieskie Źródła, an artificial lake formed by the Pilica river near Tomaszów Mazowiecki (Poland).
2. *Gammarus lacustris* G.O. Sars : ♀♀ 13–15 mm, ♂ 15–18 mm, from the peat-bog near Blonie, Lenczyca District (Poland), and
3. *Gammarus roeseli* Gerv. : ♀♀ 7–10 mm, ♂♂ 9–13 mm, from the uppermost reach of the river Noteć, near Iżbica Kujawska, Kolo District (Poland).

##### ISOPODA :

1. *Asellus aquaticus* (L.) : ♀♀ 7–11 mm, ♂♂ 9–13 mm, from ponds and streams in various localities near the city of Łódź (Poland),
2. *Oniscus asellus* L. : ♀♀ 11–14 mm, ♂♂ 10–13 mm, from a stock colony maintained at the Institute of Botany and Zoology (The University of Łódź, Poland) at 21°C and 94–100% relative humidity, on pieces of potatoes and carrots,
3. *Porcellio laevis* Latr. : ♀♀ 15–18 mm, ♂♂ 14–20 mm, collected from the various parks in the city of Prague (Czechoslovakia), and
4. *Ligia oceanica* (L.) : ♀♀ 20–25 mm, ♂♂ 16–20 mm, procured from under the stones at the beach near Bologne-sur-mer (France).

The crustaceans were dried on filter-papers for about one minute, and were bled by the method of Alikhan<sup>1</sup>.

Cellulose acetate electrophoresis was performed by a slight modification of the method described by Alikhan<sup>1</sup>. The following details are of importance : cellulose acetate strips, 11 × 2.5 cm ; buffer, veronal, pH 8.6 ;  $\mu$ , 0.05 ; current, 2.5 mA per strip ; usually four to five strips were run simultaneously ; stain, amido black ; washing in acetic acid : methanol : water ; clearing in bromonaphthalene : paraffin oil ; scanning, with Densitometer ERJ-65. Protein fractions were identified by the criteria outlined by Declair and Vercauteren<sup>3</sup>, and by Alikhan and Lysenko<sup>2</sup>.

Whenever required, peaks of individual protein bands were measured from densitometric tracings of the cellulose acetate strips by means of a polar planimeter. These units were converted to percentage of the total number of units for the entire protein pattern, and expressed as such.

#### RESULTS

As is obvious from Fig. 1, the haemolymph from almost all species showed four distinct bands of various mobilities. Fraction 1, a dense, fast moving band, when denatured by heat, displayed distinct peroxidase activity. This was considered to be the respiratory pigment, haemocyanin. This haemocyanin fraction was always followed by a relatively smaller band (fraction 4, in Fig. 1), which gave a negative lipoprotein, but a positive glycoprotein reaction.

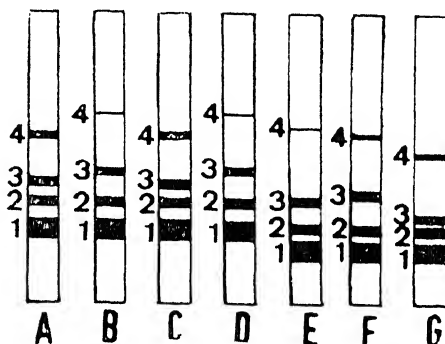


FIG. 1. Cellulose acetate electrophoretic pattern of the haemolymph proteins in various amphipod and isopod species. 1, haemocyanin ; 2 and 3, apo subunits of haemocyanin ; 4, glycoprotein ; A, *Gammarus fossarum* ; B, *Gammarus lacustris* ; C, *Gammarus roeseli* ; D, *Asellus aquaticus* ; E, *Oniscus asellus* ; F, *Porcellio laevis* ; and G, *Ligia oceanica*.

The remaining two bands (fractions 2 & 3) could not be identified with certainty. However, on the basis of their molecular weights (Alikhan & Lysenko, unpublished data), they have been regarded as apo subunits of haemocyanin.

The data on the haemolymph protein measurements in various species (Table II) revealed that major part (about 82–96%) of the plasma proteins in these species is formed by haemocyanin. According to their plasma haemocyanin contents, these seven species fall roughly into two categories : category 1, comprising of *G. fossarum*, *Oniscus asellus* and *P. laevis*, and category 2, formed by *G. lacustris*, *G. roeseli*, *A. aquaticus* and by *L. oceanica*.

Females in all species, in general, contained relatively more haemocyanin than did the males.

TABLE I  
Haemolymph coloration in various species

Species	Coloration scale	Usual colour
<i>Gammarus fossarum</i>	bluish-yellow, turning blue when exposed to air	blue
<i>Gammarus lacustris</i>	Yellowish-green, dirty green, dirty blue	brownish-green
<i>Gammarus roeseli</i>	yellow, yellowish-green, green	green
<i>A. aquaticus</i>	colourless, pale, yellow	pale yellow
<i>O. asellus</i>	colourless, pale yellow	pale yellow
<i>P. laevis</i>	light yellow, dark yellow	light yellow
<i>L. oceanica</i>	colourless, yellow	yellow

TABLE II  
Relative concentration (in percentage) of haemolymph proteins in various species

Species	Haemocyanin	Glycoprotein
<i>Gammarus fossarum</i>	95.60 ± 7.8	4.4 ± 1.2
<i>Gammarus lacustris</i>	83.52 ± 4.4	16.4 ± 0.5
<i>Gammarus roeseli</i>	83.39 ± 6.9	16.6 ± 0.9
<i>Asellus aquaticus</i>	81.30 ± 3.1	18.7 ± 0.7
<i>Oniscus asellus</i>	93.5 ± 1.7	6.5 ± 0.2
<i>Porcellio laevis</i>	94.2 ± 1.9	5.8 ± 0.2
<i>Ligia oceanica</i>	85.76 ± 5.1	14.2 ± 0.9

Average of 30-36 samples in each case ± S.E.

#### DISCUSSION

The blood in members of the class Crustacea is an important medium for the transportation of ions and molecules involved in energy metabolism. As a consequence, the composition of blood supply an important information on the physiological and pathological state of these animals.

The haemolymph proteins in several amphipod and isopod species have previously been analyzed using cellulose acetate<sup>1,11</sup>. The result of these studies demonstrated the presence of a maximum number of five protein fractions: a glycoprotein, a fibrinogen, a heteroagglutinin, an apohaemocyanin and the haemocyanin. In the present studies, the presence of fibrinogen and heteroagglutinin could not be demonstrated. Nevertheless, the present studies did show certain intraspecific differences, in spite of the fact that there were large variations among the relative contents of the various proteins

within the same species. These differences have been related to the physiological state of the animal<sup>1-2,10</sup>.

The result obtained in the present study clearly shows that in its haemolymph protein composition, *G. lacustris* is somewhat identical to *G. roeseli* on the one hand, and *A. aquaticus* and *L. oceanica* on the other. Morphologically, *G. fossarum* and *G. lacustris* have been considered to belong to the same so-called "pulex-group"<sup>8</sup>, while *G. roeseli*, as well as *A. aquaticus* and *L. oceanica*, fall into morphological categories far removed from *G. lacustris*. The implication here is that the similarity in the protein compositions in *G. lacustris* and *G. roeseli* could be due to their ecological affinity. Both of these species, along with *A. aquaticus* and *L. oceanica*, have been considered as euryoxybionts<sup>8</sup> in relation to other *Gammarus* species, since they can withstand variations in the oxygen tension of their environments. *G. fossarum* (like *Oniscus* and *Porcellio*), on the other hand, lives only in well aerated medium, and as a consequence is regarded as polyoxybiont<sup>8</sup>. This situation is also reflected by the "saprobiont system", defined by Breitig<sup>8</sup>. Under this system, *G. roeseli* has been defined as a  $\beta$ -mesosaprobiont, and *G. fossarum* as a poligosaprobiont, implying again that in their oxygen and other life requirements, these two species differ from each other.

Similarly, the protein composition similarity between *O. asellus*, *P. laevis* and *G. fossarum* imply similarity in their habitat and oxygen requirements. Both *O. asellus* and *Porcellio laevis* are terrestrial species, and as such are unable to tolerate any variation in the oxygen tension of their environments. *A. asellus*, a closely related species to *P. laevis*, is an  $\alpha$ -mesosaprobiont, which again places it in the category of *G. lacustris*.

Wieser<sup>11</sup> also contends that the haemolymph protein compositions in a given species are affected by its ecological position. According to him, the relative haemocyanin content of the haemolymph tends to become more stable as the species acquires more terrestrial habitat. However, this hypothesis needs further verification.

#### ACKNOWLEDGEMENTS

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### MORPHOGENESIS IN STEM-CALLUS TISSUE OF *CITRUS GRANDIS* IN LONG-TERM CULTURES—A BIOCHEMICAL ANALYSIS

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#### ABSTRACT

Stem-callus tissue of *C. grandis*, in long-term cultures, differentiated into two types of tissues: type-A, which was compact-nodular and slow growing and type-B, which was friable-spongy and fast growing. In a medium containing 0.25 mg/l BAP + 0.1 mg/l NAA + 500 mg/l ME, the type-A tissue produced numerous shoot-buds and shoots, whereas the type-B tissue did not. The two types of tissues also differed in respect of their nitrogen, protein, free amino acid and sugar contents.

#### INTRODUCTION

THERE are several reports<sup>1-3</sup> of the gradual loss of the regenerative capacity of plant callus tissues grown *in vitro* for a long time. Some workers<sup>4-7</sup> have studied the changing cytological conditions in the tissue during its prolonged culture and the correlated loss of its regenerative capacity. However, since callus tissue of varied ploidy including haploidy are known to differentiate organs and plantlets<sup>8-10</sup>, and those with abnormal polyploid chromosome numbers, to form abnormal shoots<sup>6</sup>, it appears that besides cytological alterations, some biochemical changes in the tissue during its prolonged culture may also be involved in the phenomenon of the loss of its morphogenetic potentiality. It seems that the latter aspect has not been studied so far, though some analyses of

free amino acids in *in vitro*-grown tissues have been made<sup>11-12</sup>. In the present investigation, certain biochemical changes in long-term culture have been studied with a view to find out any correlation between the metabolic changes in the tissue and the loss of its regenerative capacity.

#### EXPERIMENTAL PROCEDURE

Composition (in mg/l) of MS medium, a variant of Murashige and Skoog's medium<sup>13</sup>, where it differed from the liter, was: 150 NH<sub>4</sub>NO<sub>3</sub>, 1500 KNO<sub>3</sub>, 400 CaCl<sub>2</sub>, 150 KH<sub>2</sub>PO<sub>4</sub>, 360 MgSO<sub>4</sub>·7H<sub>2</sub>O, 10 thiamine-HCl, 2.5 pyridoxine-HCl, 2.5 nicotinic acid, 0.1 folic acid, 0.1 riboflavin, 0.1 biotin, 5 ascorbic acid, 50,000 sucrose and 7000 agar. Sterilization procedure and other cultural conditions were as reported earlier<sup>14</sup>. Stem-callus tissue of *C. grandis* was maintained in MS medium



supplemented with 0.25 mg/l kinetin (Kn), 0.5 mg/l  $\alpha$ -naphthaleneacetic acid (NAA) and 0.25 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) —MS-1 medium—for 2½ years and later in the same medium but devoid of Kn—MS-1a medium.

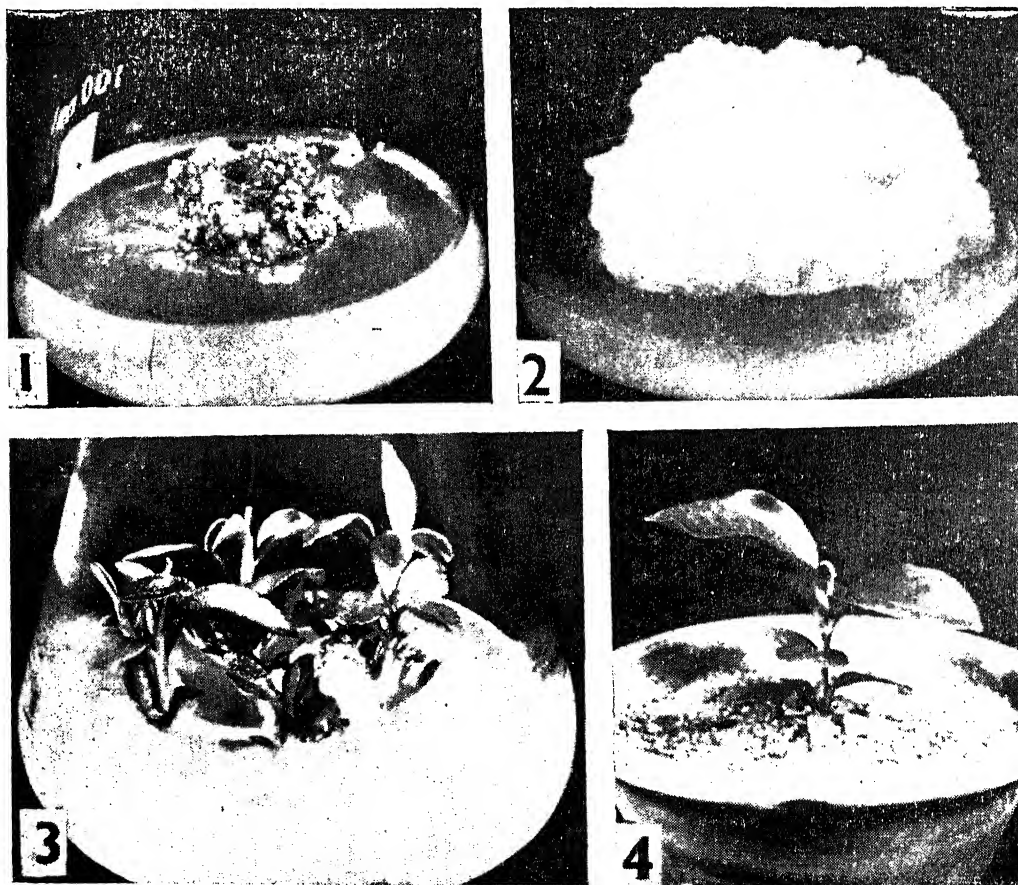
For organogenesis, the callus-tissue was cultured in MS-1b medium having half the concentrations of thiamine-HCl, pyridoxine-HCl and nicotinic acid, than present in MS medium and supplements of 0.25 mg/l 6-benzylaminopurine (BAP), 0.1 mg/l NAA and 500 mg/l malt extract (ME).

The callus tissue was analysed for quantitative determination of its nitrogen, protein, free amino acid and sugar contents. The nitrogen percentage of the callus tissue was determined by using modified Kjeldahl method<sup>15-16</sup> and the protein percentage was obtained by multiplying the former by 6.25. The quantitative determination of free amino

acids of the callus tissue was made by paper chromatography following the procedure reported elsewhere<sup>17</sup>. The sugar content of the callus tissue was estimated after the method of Dubois *et al.*<sup>18</sup>.

#### EXPERIMENTAL RESULTS

Stem-callus tissue of *C. grandis* was compact-nodular and greenish-white in colour—named as Type-A tissue (Fig. 1). After its repeated sub-culturing for 2–2½ years in MS-1 medium, the Type-A tissue in certain cultures produced a friable-spongy tissue of pale-white colour. The friable-spongy tissue was isolated and grown in separate cultures and named as Type-B tissue (Fig. 2). The Type-B tissue showed profuse growth in MS-1a medium, which was far greater than that of the Type-A tissue in the same medium (Table I).



FIGS. 1-4. Cultures of *Citrus grandis*. Fig. 1. Stem-callus tissue Type-A ( $\times 0.99$ ). Fig. 2. Stem-callus tissue Type-B ( $\times 1.11$ ). Fig. 3. Differentiation of shoots from stem-callus tissue Type-A ( $\times 1.18$ ). Fig. 4. A tissue-regenerated plant in potted soil ( $\times 0.60$ ).

TABLE I

Comparative growth in vitro of two types of stem-callus tissues of *C. grandis* in MS-1a medium

Stem-callus tissue	Wt. of inoculum*		Growth of explant*					
			10 days		20 days		30 days	
	Fr. wt. (gm)	Dry wt. (mg)	Fr. wt. (gm)	Dry wt. (mg)	Fr. wt. (gm)	Dry wt. (mg)	Fr. wt. (gm)	Dry wt. (mg)
Type-A	0.4	60.16	0.4	68.86	0.6	77.84	0.8	125.72
Type-B	0.5	19	0.95	41	4.3	196.63	9.9	537.5

\* Average of 5 replicate cultures.

In MS-1b medium, 2 to 2½-year-old Type-A tissue produced a large number of green shoot-buds which all developed into shoots (Fig. 3). It took about 60 days for shoot-buds to be visible on the callus surface and in the following 30 to 40 days they developed into shoots. Roots were not produced by the differentiating callus cultures. However, isolated tissue-regenerated shoots could easily be rooted in a different medium and were made to develop as complete plants in potted soil (Fig. 4). On the contrary, the Type-B tissue did not form any shoot-buds in the same medium, though it became a bit less friable. Also, roots were not produced by the Type-B tissue. Same results were obtained by repeating the experiment for several times.

Stem-callus tissue: Type-A and Type-B not only showed differences in their morphology, growth rate and morphogenic potentiality, but also in their biochemical make-up in respect of nitrogen, protein, free amino acid and sugar contents, as presented in Table II. Concentrations of the chemical constituents assayed, *viz.*, nitrogen, protein, free amino acids and sugars were considerably less in Type-B tissue than in Type-A tissue. With respect to free amino acid content of the two types of tissues, they differed not only quantitatively, but also qualitatively, *i.e.*, fewer amino acids were present in the Type-B tissue. In all, 11 amino acids were present in Type-A tissue, whereas only 7 in the Type-B tissue. L-Glutamic acid, L-proline, L-serine and L-tyrosine were not present in the Type-B tissue. Asparagine, L-glutamic acid and glycine were the prominent amino acids in the Type-A tissue, whereas L-arginine, glycine and L-tryptophane in the Type-B tissue. Amongst the common amino acids present in both types of tissues, the decrease in their concentrations in the Type-B tissue with respect to Type-A tissue was most pronounced with L-asparagine, followed by L-threonine, L-aspartic acid, glycine, L-tryptophane,

TABLE II

Quantitative analysis of two types of stem-callus tissue of *C. grandis* for their nitrogen, protein, free amino acid\* and sugar\* contents

Chemical constituents	Stem-callus tissue	
	Type-A	Type-B
1. Nitrogen	1.337 %	0.5954 %
2. Protein	8.356 %	3.722 %
3. Amino acids	L-Alanine	16.6 9.6
	L-Arginine	18.7 16.4
	L-Asparagine	45.8 10.7
	L-Aspartic acid	32.0 12.0
	Glycine	38.2 14.7
	L-Glutamic acid	42.0 ..
	L-Proline	16.0 ..
4. Sugars	L-Serine	20.5 ..
	L-Threonine	25.0 8.02
	L-Tryptophane	31.6 14.1
	L-Tyrosine	12.2 ..
	D-Glucose	198.0 62.3
	Sucrose	99.0 50.4
	Fructose	81.0 39.0

\* Quantity in µg/100 mg fr. wt. of callus tissue; data based on three chromatographs of each sample.

L-alanine and L-arginine. Amongst sugars, D-glucose was the most prominent in both the types of tissues, followed by sucrose and fructose.

#### DISCUSSION AND CONCLUSION

During prolonged culturing, the plant tissues are known to undergo many kinds of changes and not infrequently new strains have been obtained differing in one or more such characteristics as texture, compactness, colour, growth rate, growth requirements, and morphogenetic potentiality. To cite a few examples: in cultures of carrot, strains with compact type of growth resulted from highly friable parent strain<sup>19</sup>, callus strains of *Trichocereus* differed in growth rates and texture<sup>20</sup>, whereas those of *Melilotus* and *Opuntia* calli in pigmentation<sup>21</sup>. Similarly, in the present case, during prolonged cultures of stem-callus tissue of *C. grandis*, a new type of tissue named Type-B, which was friable-spongy, pale-white and fast growing, resulted from parent Type-A tissue characterized by being compact-nodular, greenish-white and slow growing.

In contrast with the situation obtained in long-term cultures of pea root-callus, where the callus strain of hard texture and reduced growth rate, originated from friable and comparatively fast growing parent tissue, lost the organogenetic capacity<sup>5</sup>, the friable and fast growing type of citrus tissue, i.e., Type-B derived from compact-nodular and slow growing tissue, did not form organs. This tissue (Type-B) lacking regenerative potentiality not only had strikingly less concentrations of free amino acids, nitrogen, protein and sugars than that found in the potentially differentiating tissue, i.e., Type-A, but also lacked certain amino acids altogether, viz., L-glutamic acid, L-proline, L-serine and L-tyrosine. Thus in the present case, the loss of regenerative potentiality seems to be linked with fast growth and certain concurrent changes in the biochemical composition of the tissue.

#### ACKNOWLEDGEMENT

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Project No. A7-CR-286. We thank the Scientist in-Charge, National Botanic Gardens, Lucknow, for the facilities provided.

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## LETTERS TO THE EDITOR

### A NOTE ON THE VARIATION OF THE FREEZING LEVEL OVER INDIA

PRAMANIK AND KOTESWARAM<sup>1</sup>, using aircraft data for the first time, presented the frequencies of occurrences of low clouds. They have pointed out that 90% of the low cloud tops lie below freezing level during the year. The percentage frequency of clouds reaching above freezing level being least in the South-West monsoon season, they have stated that the mean freezing level in winter season over India falls to 10,000 feet over North India and slopes upwards to 15,000 feet in South India. It is fairly steady all over the country in the monsoon season

summer monsoon season, the freezing level is uniform within the height of 5.2 km between 16° N to 31° N latitudes. The clouds that are followed within this height are warm clouds and require accordingly suitable techniques for seeding them, either to increase or control the rainfall.

The mean monthly freezing levels over 14 radio-sonde stations in India for both synoptic hours are taken from the Monthly Weather Reviews' data for the year 1965. Published by India Meteorological Department. The mean of the freezing levels of both synoptic hours is found out and presented in Table I. The height of the freezing level

TABLE I

Sl. No.	Name of the Station	J	F	M	A	M	J	J	A	S	O	N	D
1.	Ahmedabad	4.0	4.0	3.3	3.4	4.8	5.4	5.5	5.5	5.3	4.9	4.7	4.7
2.	Allahabad	3.7	3.3	3.7	4.4	4.5	5.1	5.5	5.4	5.1	4.6	4.3	4.1
3.	Bangalore	4.8	4.9	4.9	4.9	5.1	5.2	5.1	5.2	5.2	5.2	5.3	5.2
4.	Bombay	4.5	4.5	4.5	4.8	5.0	5.3	5.3	5.3	5.2	5.1	5.2	5.2
5.	Calcutta	4.0	3.8	4.1	4.5	4.8	5.1	5.5	5.3	5.2	4.7	4.5	4.5
6.	Gauhati	3.7	3.4	3.8	4.5	5.0	5.7	5.7	5.4	5.4	4.7	4.2	4.4
7.	Jodhpur	3.6	3.4	3.8	4.2	4.6	5.1	5.6	5.4	5.5	..	..	..
8.	Madras	4.7	4.8	4.9	5.0	5.1	5.1	4.9	5.1	5.1	5.2	5.0	5.2
9.	Nagpur	4.2	4.0	4.2	4.6	4.8	5.2	5.3	5.4	5.3	4.8	4.8	4.4
10.	New Delhi	3.3	3.1	3.6	4.2	4.4	4.9	5.5	5.3	5.1	4.5	4.3	3.8
11.	Port Blair	4.6	5.4	5.0	5.0	5.4	4.9	5.0	5.2	5.3	5.2	5.1	5.0
12.	Srinagar	1.6	1.6	2.5	3.7	4.0	4.6	4.9	4.8	4.2	3.7	3.2	1.6
13.	Trivandrum	4.9	4.7	5.0	5.0	5.0	4.9	5.0	5.0	4.9	5.0	5.0	5.1
14.	Visakha- patnam	4.5	4.6	4.5	4.8	5.0	5.3	5.1	5.3	5.3	5.1	4.9	4.9

at about 16,000 feet to 18,000 feet. This type of information is very useful in deciding cold and warm clouds. This information is useful in designing the cloud seeding experiments. The authors in the present work have presented the seasonal and latitudinal variations of the freezing level over radio-sonde stations in India with more reliable data. It is observed by the authors, that during

is lower in winter, in comparison with summer, over all the stations (Fig. 1). The increase in height from winter continues till the South-west monsoon season, after which it decreases during the post-monsoon season. Thus the annual cycle of the variation of the height of freezing level consists of one maxima during the South-west monsoon season and two minima in

winter and Post-monsoon seasons. The variations of height over higher latitudes are more prominent than over lower latitudes (Fig. 3). Over inland stations, the variations are more conspicuous than over coastal stations. The variations of height of the freezing level is more in winter than in summer (Fig. 2). Thus in the South-west monsoon season, the level is steady and uniform.

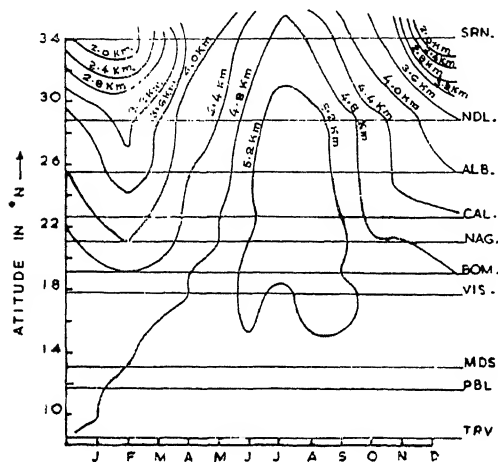


FIG. 1. Cross-section of the seasonal and latitudinal variation of the freezing level around 80° E longitude.

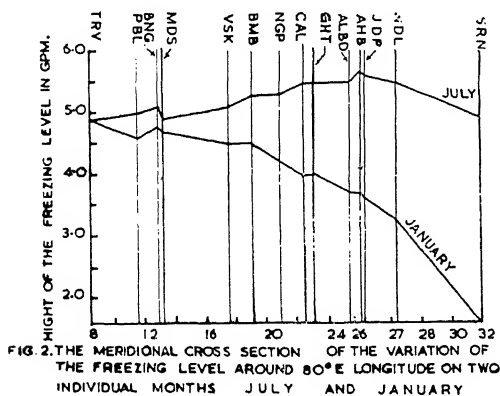


FIG. 2. THE MERIDIONAL CROSS SECTION OF THE VARIATION OF THE FREEZING LEVEL AROUND 80° E LONGITUDE ON TWO INDIVIDUAL MONTHS JULY AND JANUARY

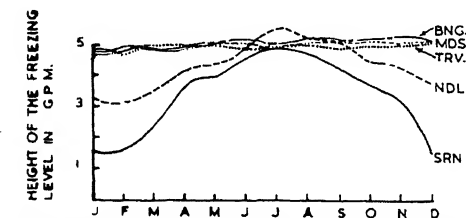


FIG. 3. VARIATIONS OF THE FREEZING LEVEL OVER INLAND, COASTAL AND HILL STATIONS.

The above-said information is important in designing the cloud seeding experiments. For increase of rainfall during South-west monsoon season, the warm cloud seeding technique is suggested.

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Oceanography, J. K. PATNAIK.  
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Waltair, January 7, 1974.

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### A NOTE ON MONTHLY MEAN MAXIMUM MIXING HEIGHTS

THERE are certain parameters like gustiness, etc., by which the air pollution potentialities of a locality can be measured. Monthly mean maximum mixing height is one such parameter, which gives climatologically the pollution potentialities of a place. The monthly mean maximum mixing heights have been computed for ten Indian stations for twelve months. The method of computation given by G. C. Holzworth (1964) has been adopted to compute the mixing heights. The necessary data to compute these values are taken from climatic tables and climatic normals published by Indian Meteorological Department. The preliminary results of the analysis are discussed in this note.

From Table I, it is observed that the monthly mean maximum mixing heights, on an average, are high in summer, low in winter and least in the monsoon season. In summer season, because of strong solar insolation, the lower layers of the atmosphere are kept in a high turbulent state and hence their mixing into a greater depth of the air column is possible. But, the lower layers of the atmosphere, more or less, are kept in a stable condition in winter; the mixing heights are low in this season. Since the mixing heights are also dependent on the temperature contrast between maximum and the temperature at the time of morning ascent, the heights are least in monsoon season, as the temperature contrast is minimum during this period due to high cloud amount and frequent occurrence of rainfall.

The monthly variations of mixing heights of an inland station are higher than a coastal station. The heights for coastal stations are maintained constant throughout the year with little deviations. The above-mentioned reason, namely, the "temperature contrast" can be attributed to these variations, it being low from month to month. In addition to this the land and sea breeze, and orographic effects also contribute considerable influence on vertical mixing.

TABLE I

Mean monthly maximum mixing heights for some Indian stations  
(Meters)

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Ahmedabad	.. 1,550	1,610	2,160	2,620	2,240	1,800	1,120	970	1,310	1,950	1,940	1,510
Bangalore	.. 1,380	1,850	2,320	2,180	2,070	1,390	1,200	1,240	1,270	1,220	1,160	1,010
Bombay	.. 1,390	1,280	1,110	900	760	790	600	670	820	1,150	1,380	1,560
Calcutta	.. 1,600	1,590	1,580	1,360	1,100	750	810	820	1,030	1,280	1,600	1,840
Madras	.. 1,300	1,180	1,130	1,290	1,550	2,130	1,800	1,790	1,390	1,240	940	1,280
Nagpur	.. 1,500	2,050	2,940	2,940	3,400	2,260	1,020	990	1,170	1,390	1,580	1,730
New Delhi	.. 1,200	1,100	1,360	1,850	2,200	1,390	1,030	1,270	1,440	1,710	1,650	1,270
Poona	.. 1,750	2,150	2,970	2,470	1,890	1,410	820	930	1,280	1,500	1,410	1,450
Trivandrum	.. 1,540	1,500	1,420	1,140	1,110	840	620	1,020	1,020	1,050	1,170	1,320
Visakhapatnam	.. 1,030	980	750	750	730	730	860	960	570	1,050	1,210	1,030

Maximum value of mean monthly maximum mixing height occur in the month of May at Nagpur; the value being 3.4 kilometers and minimum at Visakhapatnam in the month of December (0.57 km). In most of the months, Visakhapatnam is showing low mixing heights compared to other stations. Bombay is showing low mixing heights in summer and high mixing heights in winter season. Except Madras, other coastal stations are also showing similar type of distribution. Poona, in spite of its proximity to the sea coast, is showing mixing height distribution, similar to that of a continental station, because of orographic influence.

The authors express their gratitude to Prof. R. Ramanadham, Head of the Department of Meteorology and Oceanography, Andhra University, Waltair, and Project Manager, WMO/UNDP, Nairobi, Kenya, for facilities to carry out this work and constant guidance and encouragement.

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#### SPECTROPHOTOMETRIC STUDIES ON IRON(III) COMPLEXES

THE complexes of iron(III) with orthohydroxy ketones have been studied by various workers using spectrophotometric methods<sup>1-4</sup>. Iron(III) has been shown to form coloured 1:1 complexes with these ketones at low acidity. This fact suggests the possibility of using these ketones as auxiliary ligands in spectrophotometric investigation of colourless complexes of iron(III). We have reported earlier<sup>5</sup> the use of 2-hydroxy-5-methylbutyrophenone in determining the stoichiometry of exalate and ethylenediaminetetraacetate complexes of iron(III). The present paper describes the results on composition and stability of the chelate of iron(III) with 2-hydroxy-5-chlorobutyrophenone (5-ClOHB). It also describes the use of the same reagent as an auxiliary ligand in determining the composition of the colourless complexes formed by citric, oxalic, malonic and ethylene diamine tetra-acetic acids with iron(III) in acid media.

*Experimental.*—5-ClOHB (M.P. 47–48°) was prepared by Fries migration of 5-chloro-phenylbutyrate using anhydrous aluminium chloride in absence of a solvent. Standard reagent solutions were prepared by dissolving weighed amounts of the reagent in pure methanol.

All the other chemicals used were of A.R. quality. The stock iron(III) solution was prepared as shown earlier<sup>5</sup>. The stock solutions of carboxylic acids were prepared by dissolving a calculated amount of

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each in 0.01M  $\text{HNO}_3$ . Dilute solutions of same acidity but desired concentration were obtained as and when needed by diluting the stock solutions with 0.01M  $\text{HNO}_3$ . All aqueous solutions were prepared in water twice distilled over alkaline permanganate.

Spectrophotometric measurements were made on Zeiss-Specol, using 1 cm matched cells. All measurements were carried out at  $30 \pm 1^\circ \text{C}$  in a 50% methanol-50% water (by volume) medium adjusted to 0.005 M  $\text{HNO}_3$ .

**Results and Discussion.**—The method of Vosburgh and Cooper<sup>6</sup> was employed to determine the nature of complexes. Mixtures containing 1:1, 1:2 and 1:3 mole ratios of iron(III) to 5-CIOHB were prepared. Absorbance measurements were carried out between 400 nm and 700 nm. All the mixtures showed maximum absorbance at the same wavelength of 535 nm thus indicating formation of a single complex. All the subsequent absorbance measurements were carried out at 535 nm. The composition of the complex was determined by two different methods, Job's method<sup>7</sup> using equimolar solutions and the slope-ratio method<sup>8</sup>. It was found to be 1:1 in both cases. The apparent stability constant of the complex  $\log K = 2.39 \pm 0.1$  was calculated from the absorbance data by the method of Mukherjee and Dey<sup>9</sup>.

**Colourless Complexes of Iron(III).**—The method used has been described earlier<sup>5</sup>. A solution containing  $\text{Fe}(5\text{-CIOHB})^{+2}$  ( $1 \times 10^{-3} \text{ M}$ ) was prepared by mixing equal volumes of iron(III) ( $2 \times 10^{-3} \text{ M}$ ) and 5-CIOHB ( $1 \times 10^{-2} \text{ M}$ ) solutions. A series of mixtures was then prepared by continuous variation method<sup>7</sup>, using  $\text{Fe}(5\text{-CIOHB})^{+2}$  ( $1 \times 10^{-3} \text{ M}$ ) and carboxylic acid ( $1 \times 10^{-3} \text{ M}$ ) solutions. The absorbances were measured at 535 nm.

Two sets of solutions for each system, one with and other without the above carboxylic acids, were prepared. The difference in the absorbance for the two sets corresponds to the y function in Job's curve. Maximum in y would indicate the composition of the colourless complex. Typical results are shown in Fig. 1 for the  $\text{Na}_2\text{EDTA}$  system. It can be seen that the maximum decolorization occurs at a ratio of iron(III):  $\text{Na}_2\text{EDTA} = 1:1$ . This indicates the formation of a 1:1 complex,  $\text{Fe}(\text{EDTA})^-$  under the experimental conditions. Results obtained with citric acid, oxalic acid and malonic acid also indicated that iron(III) forms 1:1 complexes with these acids under the conditions of the present study.

In order to find out whether mixed ligand complex formation occurs in these systems, the follow-

ing test was done. Increasing amount of carboxylic acid was added to a solution containing iron (III) and a large excess of 5-CIOHB. The observation of a continuous decrease in the absorption over the entire visible range with increasing concentration of the added acid was interpreted as progressive conversion of iron(III)-5-CIOHB to iron(III)-carboxylate complex. It was concluded

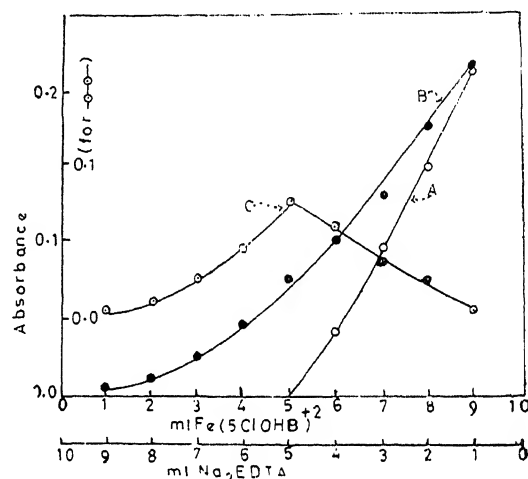


FIG. 1. Job's curves in equimolar solution for iron(III)-EDTA complex. Curve A:  $\text{Fe}(\text{III})$ -5-CIOHB- $\text{Na}_2\text{EDTA}$ . Curve B:  $\text{Fe}(\text{III})$ -5-CIOHB. Curve C: Difference of curves A and B.

therefore that mixed ligand complexes were not formed, at least under the conditions of present study, in these systems.

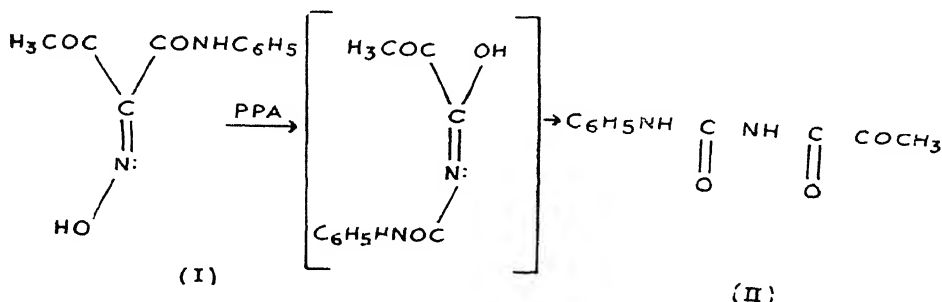
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# REARRANGEMENT OF ISONITROSOACETO- ACETANILIDE USING POLYPHOSPHORIC ACID TO SUBSTITUTED UREA\*

POLYPHOSPHORIC acid (PPA) is a very effective reagent for cyclization-dehydration reactions<sup>1</sup>. The cyclization of malon-monoarylamides to 2, 4-dihydroxyquinolines was described by Patel and Mehta<sup>2,3</sup>. The rearrangement of 3, 5-dimethyl-2-cyclohexene-1-oxime to cyclic amide with PPA has been reported by Horming<sup>4</sup>.



It appeared of mechanistic interest to study the action of PPA on isonitrosoacetoacetanilide (I). In principle the reaction could give isatin or 4-acetyl-2-hydroxy quinazoline depending upon the *syn* or *anti* structures of the oxime.

It has, however, been found that treatment of (I) with PPA at temperatures ranging from 95–130° did not give either of these products and instead substituted urea (II) is formed which can be rationalized as shown above :

The structure assigned to the urea is based on analysis, hydrolysis products and chemical reaction studies. This is a general reaction as the reaction took the same course with a number of anilides of type I. The rearrangement is probably initiated by acid catalysis.

**Experimental.**—To the solution of PPA (81–85%) prepared from 10 g of  $P_2O_5$  and 6 ml  $H_3PO_4$  was added 2 g (0.01 mole) of isonitrosoacetoacetanilide and the reaction mixture heated for 1 hr at 100°. The temperature was then raised to 130° and heating continued for another 2 hr. The reaction mixture was decomposed by pouring into cold water and the solution was made slightly alkaline. The product was filtered, washed and dried. The material on recrystallisation from benzene gave 1.5 g (75%) of a product; m.p. 220°. (Found: N, 13.6;  $C_{10}H_{10}N_2O_3$  requires N, 13.58%).

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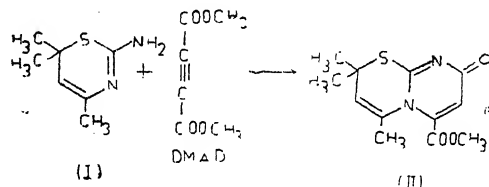
\* This work was carried out by the author at Chemistry Department, University of Baroda, India.

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## REACTION OF DIMETHYL ACETYLENECARBOXYLATE WITH 2-AMINO-4, 6, 6-TRIMETHYL-1, 3-THIAZINE

DIMETHYL acetylenedicarboxylate, one of the most reactive dienophiles, has played an important role in organic synthesis<sup>1</sup>. It undergoes a wide variety of 1, 3-dipolar cycloadditions<sup>2</sup>, Diel's Alder type of additions<sup>3</sup> and also is easily attacked by several nucleophiles giving rise to a variety of products<sup>4</sup>. The reaction of acetylenic esters with heterocyclic compounds have been the subject of several publications in recent years.

The present communication reports the addition of dimethyl acetylenedicarboxylate (DMAD) to 2-amino-4, 6, 6-trimethyl-1, 3-thiazine (I)<sup>5</sup> to yield the cyclised product (II) with elimination of one molecule of methanol. The structure of the product was established on the basis of various spectral and analytical data.



DMAD was added to a stirred solution of 2-amino-4, 6, 6-trimethyl-1, 3-thiazine in ether at 0° C. The reaction mixture was stirred further for 2 hours. A bright yellow coloured solid was obtained which



was recrystallised from methanol to yield pure (II) m.p. 118° C (Yield, 72%).

(Found : C, 54.03; H, 5.29; N, 10.41; S, 12.18.  $C_{12}H_{14}N_2SO_3$  requires C, 54.12; H, 5.26; N, 10.52; S, 12.03%). Molecular weight = 266. U.V. spectrum (Ethanol,  $nm$ : 390, 304, 256. I.R. (Nujol,  $cm^{-1}$ ) 1740  $s$  (ester), 1705  $s$  (C=O), 1660  $s$  (C=N), 1260  $s$  (C=N), 1200  $s$ , 1155  $s$ , 1120  $m$ . NMR (60Hz,  $CDCl_3$ , TMS, values): 6.3  $s$  (1H, olefinic proton adjacent to ester group), 5.45  $d$  (1H, olefinic proton adjacent to methyl group,  $J = 1$  cps), 3.9  $s$  (3H,  $-OCH_3$ ), 1.95  $d$  (3H,  $-CH_3$ )  $J = 1$  cps), 1.7  $s$  (6H, two  $-CH_3$ ).

The author is thankful to Dr. H. S. Sachdev for valuable guidance and Prof. G. B. Singh for providing necessary facilities and to the C.S.I.R., New Delhi, for financial help.

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Banaras Hindu University,  
Varanasi-5, January 1, 1974.

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#### GRAVIMETRIC DETERMINATION OF CADMIUM WITH N-BENZOYL- o-TOLYLHYDROXYLAMINE

N-BENZOYL-*o*-TOLYLHYDROXYLAMINE has recently been used in the determination of certain metals<sup>1-3</sup>. The purpose of the present communication is to investigate further uses of this reagent for analytical problems.

**Experimental.**—All the reagents were of AnalaR grade. The ligand was prepared by the method as described earlier<sup>1</sup>. The ligand solutions were made by dissolving it in 10–20 ml of 90% ethanol before use. Standard cadmium (II) solution was prepared from cadmium nitrate and standardized by the usual method<sup>4</sup>. Solutions of other ions were prepared from nitrate or the sulphate salts of cations and from the sodium, potassium and ammonium salts of anion. A Cambridge pH meter was used for pH measurements.

**Procedure.**—An aliquot of cadmium (II) solution containing 5.0 to 32.0 mg of the metal was diluted to 200 ml, heated to 40–50° C and the reagent (three-fold) solution was added with stirring. The pH of the mixture was then raised

to 5.8–7.0 by the addition of dilute sodium hydroxide solution. A white precipitate of cadmium complex that formed was made to flocculate by digesting on the hot water-bath (60–70° C) for 20 to 30 minutes with occasional stirring. It was filtered through a weighed medium porosity sintered glass crucible, washed with hot (50° C) water, dried at 110–120° C to a constant weight (1.3 hours), and finally weighed as  $Cd(C_{14}H_{12}O_2N)_2$ . The gravimetric factor (cadmium/cadmium complex) is 0.1991. The results are given in Table I.

TABLE I  
Determination of cadmium with the reagent

Mg. metal Taken	Mg. metal Found	Error %
	5.11	–0.19
5.12	5.12	0.00
	12.36	–0.32
12.40	12.41	+0.08
	30.06	+0.13
30.00	30.10	+0.33

Determination of cadmium in presence of other ions. Co-precipitation studies were made with a number of other ions. The results indicated that Ca, Sr and Ba did not interfere. The interference of Mg, As (III), Sb (III), Mo (VI) and W (VI) was eliminated by using sodium potassium tartrate (2.0 g), while ammonium acetate (2.0 g) and ammonium carbonate (2.0 g) were used to mask La (III) and U (VI) respectively. The pH was maintained between 6.5 and 7.0 while effecting above separations and the initial washing was carried out with hot water containing a little of the masking agent. Hot water was used for the final washing. Tartrate did not interfere above pH 6.5 while oxalate, citrate, cyanide, iodide, chloride, fluoride and EDTA interfered within the pH range, 5.8 to 7.0.

**Results and Discussion.**—The white cadmium (II)-benzoyl-*o*-tolylhydroxylamine complex was sparingly soluble in acetone, ethanol, methanol, ether, benzene, chloroform, carbontetrachloride and petroleum ether. It melted with decomposition at  $221 \pm 1^\circ C$ . The complex was analysed for its carbon, hydrogen and nitrogen content to determine its composition. The composition of the complex corresponds to the formula  $Cd(C_{14}H_{12}O_2N)_2$  in which cadmium to ligand ratio is 1 : 2 (Found :

C, 59.24; H, 4.21; N, 5.00. Calc: C, 59.53; H, 4.25; N, 4.96%).

In a series of experiments the pH was fixed at 6.0 and the amount of the reagent was varied. It was observed that at least three times the theoretical quantity of reagent was necessary for complete precipitation.

In order to find out the suitable pH range in which cadmium could be determined, a series of estimations were carried out at various pH-values and it was observed that cadmium is quantitatively precipitated in the pH range, 5.8 to 7.0. The precipitation commenced at pH 4.8.

The results of a few estimations of various quantities of cadmium indicated that 5.0 to 30.0 mg of cadmium could be determined in a volume of 200 ml with an accuracy better than  $\pm 0.5\%$ .

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## INHERITANCE OF POLLEN CONTENT IN RICE

THE development and expression of different parts or organs of plants have been known to be under genotypic control. Investigations in this Institution revealed that pollen content (number of pollen grains per anther) is a varietal or genetic character in rice<sup>1</sup>. In view of the importance of pollen production in pollination, it is of significance to determine the nature of genetic control and inheritance of pollen content in rice. An experiment was carried out with eighteen varieties of rice and their hybrids to understand the nature of genetic inheritance. The results are presented in this report. This is a preliminary report indicating segregation for pollen content in plants.

Eighteen varieties of rice listed in Table I were grown in the Paddy Breeding Station, Coimbatore, and hybridisation was effected between 32 selected combinations during 1972. Parents and hybrids were sown in rows during the main season, 1972. Among the parents Co. 2, Co. 7, Adt 8 & T. 2750 have not been studied since they did not flower due to very late planting. When the plants were

in shot blade stage, 5 to 10 spikelets from each panicle were selected at the rate of ten plants from each parent and hybrid. The spikelets were then fixed in 70% alcohol in specimen tubes and stored in a frigidaire at 16° C. The spikelets from each parent or hybrid were pooled and 50 anthers were then dissected out from them and transferred to a glass vial with 1.25 ml of distilled water. A uniform suspension of pollen was prepared by gently crushing the anthers to empty the contents. One drop of Teepol was added to the suspension. The suspension was transferred by means of a micropipette to the Spencer haemocytometer. Number of pollen grains in the four corners in the haemocytometer was counted under a microscope. The count from each corner was treated as one replication. Fifty such counts were taken. The number of pollen grains was then estimated following the method of Oberle and Goertzen<sup>2</sup>. The data on mean number of pollen per unit area (corner) of haemocytometer for parents and hybrids are presented in Table I.

The number of pollen grains in the parents ranged from 1.87 to 13.12 per unit area with a mean value of 6.78. In the hybrids the range was from 3.08 to 16.87 with a mean of 6.87. Statistical analysis revealed that there was significant differences for pollen content among the parents and also among the hybrids. Among the hybrids tested, 13 had pollen content on a par with that of the higher parent and only 2 were significantly superior over the respective higher parent. Seven hybrids had pollen content significantly lower than the respective higher parent.

Two  $F_2$  populations, one from self-fertilisation of  $F_1$  of IR.20  $\times$  IR.8 that showed the lowest pollen content (3.08) and one from the  $F_1$  of PVR.1  $\times$  IR.20 that showed the highest pollen content (16.87) were studied during 1973. In each, pollen content was estimated in 40  $F_2$  plants along with parents. The results are shown in Table II. In the  $F_2$  of IR.20  $\times$  IR.8, the  $F_1$  was towards lower parent in respect of pollen content while the  $F_1$  of PVR.1  $\times$  IR.20 had surpassed both the parents for pollen content. However, in  $F_2$ s of both combinations, the range of pollen content was similar (6 to 18) and the mean values were also more or less equal.

The present investigation thus brings out that pollen content is under gene controlled systems in that different varieties differed in their pollen content such as high and low. In their crosses, some showed increase over parents while some others showed decrease in pollen content and tended towards lower parent. The  $F_2$  showed genetic segregation in a pattern characteristic with segregants being either low or medium or high in

TABLE I

*Mean pollen content per unit area in parents and hybrids in rice*

Parents	Mean number*	Hybrids	Mean number*	Hybrids	Mean number*
CO.19	4.87	PVR.1 × IR.20	16.87	CO.19 × IR.20	9.12
CO.20	8.92	IR.8 × CO.25	5.37	CO.2 × Karuna	8.66
Karuna	6.79	IR.20 × T.2750	4.25	ADT.8 × IR.8	5.91
IR.8	3.12	CO.7 × IR.8	4.62	DGWW × ADT.2	5.37
DGWW	12.33	IR.20 × CO.2	7.54	CO.2 × IR.8	8.20
Rascadam	6.66	CO.17 × IR.8	3.08	Rascadam × TN.1	4.57
T.N. 1	1.87	ADT.8 × IR.20	5.75	BAM.3 × IR.20	6.92
BAM.3	6.50	IR.20 × CO.25	6.50	Rascadam × M.40	6.50
M.40	5.42	ASD.5 × IR.22	9.58	CO.32 × IR.22	7.79
CO.32	4.00	ADT.8 × Manila	5.71	Rascadam × DGWW	10.42
ASD 11	7.91	ASD.11 × Karuna	4.50	ASD.11 × IR.8	13.16
ADT 2	5.46	CO.2 × IR.20	7.37	ADT.2 × Karuna	5.96
CO.25	6.62	ADT.22 × IR.8	3.12	CO.7 × IR.8	4.62
PVR.1	6.50	CO.2 × Manila	6.33	CO.30 × IR.20	5.29
GEB.24	12.33	ASD.5 × IR.8	11.17	GEB.24 × IR.22	4.17
ASD.5	13.12	IR.20 × CO.2	7.54	IR.20 × IR.8	3.08
Manila	5.20				
IR.20	8.90	Mean : Parents — 6.78; Hybrid — 6.87; S.E.D. — 1.59; C.D. — 3.12			

\* The mean number multiplied by 250 gives pollen content per anther.

TABLE II

*Segregation for pollen content in F<sub>2</sub>*

Details		Mean pollen content	Frequency distribution in F <sub>2</sub>						Mean
			6·01 8·00	8·01 10·00	10·01 12·00	12·01 14·00	14·01 16·00	16·01 18·00	
IR.20	..	8·90							
IR.8	..	3·12							
PVR.1	..	6·50							
IR.20 × IR.8	..	3·08	4	10	13	5	7	1	11·2
PVR.1 × IR.20	..	16·87	3	8	9	12	5	3	11·8

pollen content. In the microsporogenesis in rice one PM cell gives rise to four pollen grains. The differences in pollen production potential of a variety is also directly attributable to the number of PM cells per anther. Further, the differences in cell initials at organ levels in varieties which are governed by gene systems also might contribute towards the differences in pollen content in anther. The variation in PM cells between diploids and their autotetraploids in rice in respect of pollen content was shown to be genetically governed by Sree Rangasamy and Raman<sup>1</sup>. This present

report indicates the need to investigate in detail the inheritance pattern of pollen content.

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ISOLATION OF SEED-BORNE MICROFLORA  
FROM LEGUMINOUS CROPS AND THEIR  
ANTAGONISTIC EFFECT ON RHIZOBIUM

IN legume—*Rhizobium* symbiosis the success or failure of a particular introduced strain depends not only on the ability of the strain to infect and form nodules but also on the properties of ecosystem. In many cases, antagonistic soil<sup>1-3</sup> and seed<sup>4-7</sup> microflora have been incriminated for the failure of the expression of the introduced strain. With this in view it was thought to isolate bacteria, fungi and actinomycetes from the different leguminous crops, viz., mung (*Phaseolus aureus*), urid (*Phaseolus mungo*) and soybean (*Glycine max*) and to see their antagonistic effect, if any, on the growth of the *Rhizobium* of their respective crop. Four varieties of mung (Baisakhi, S-8, Hybrid-45 and Kopergaon) as well as four of urid (Mash-1-1, No. 41-43, Pusa-1 and MK-18) were used to see if varieties differed in harbouring the microflora.

The bacteria, fungi and actinomycetes were isolated on the nutrient agar<sup>8</sup>, rose Bengal agar<sup>9</sup> and Ken knight's agar medium, respectively. The antagonistic effect of the microflora of a particular crop was seen by streaking its isolates on yeast extract mannitol agar plates seeded with the *Rhizobium* specific to that crop. The formation of inhibition zone around the isolates was taken as criterion for its (their) antagonistic effect towards that particular *Rhizobium*.

Table I summarizes the number of isolates from each crop and also the number of antagonistic organisms. It can be seen from the results that seeds of the legumes were found to harbour different types of bacteria, fungi and actinomycetes. The number of isolates not only varied from crop to crop but also from one variety to another in the same crop, e.g., no fungal isolate was obtained from variety Kopergaon (mung). It was interesting to note that while there were some bacterial and actinomycete isolates from all the three crops which showed antagonism to their respective *Rhizobium*, no fungal isolate was found to be antagonistic in case of soybean and urid except one from each variety in case of mung.

From the table it is clear that every crop had some isolates which showed antagonistic effect on the growth of *Rhizobium*, though their numbers were less. Since these observations have been made in *in vitro* conditions, it is also possible that such antagonists are ineffective when the seed is sown in soil. On the other hand, there may be other organisms which might become antagonistic in natural conditions but were not found to

TABLE I

Number of seed borne bacteria, fungi and actinomycetes antagonistic to rhizobia (figures in parenthesis represent the total isolates)

Organism Seeds of	No. of antagonistic isolates		
	Bacteria	Fungi	Actinomycete
<i>Soybean</i>			
Clark-63	(25)/1	(5)/..	(4)/..
<i>Urid</i>			
Mash-1-1	(23)/1	(3)/..	(10)/2
No. 41-43	(24)/..	(4)/..	(2)/1
Pusa-1	(23)/1	(3)/..	(8)/4
MK-18	(29)/3	(5)/..	(7)/..
<i>Mung</i>			
Baisakhi	(22)/2	(7)/1	(6)/3
S-8	(16)/1	(3)/1	(6)/2
Hybrid-45	(15)/..	(2)/1	(7)/1
Koperagon	(17)/2	..	(10)/..

be so in *in vitro* studies.. Hence, it is still to be determined as how these effects persist under *in vivo* conditions.

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# LIGHT REGULATED DISTRIBUTION OF PHOTOSYNTHATES TO VARIOUS PLANT PARTS—AN ADAPTIVE MECHANISM\*

THE photosynthetic plant growth has been a subject of considerable interest since long. Briggs *et al.*<sup>1</sup> introduced the concept of growth analysis over half a century ago and during this period it has been much improved upon and widely used in studies of primary production<sup>2</sup> and as a tool to study effects of various environmental factors on plants<sup>3</sup>. The method has helped in understanding the adaptive mechanisms in plants to stress factors. Light intensity can be singled out as the most investigated factor<sup>4</sup>. Whitehead<sup>5</sup> concluded that the species which maintain themselves successfully at less than full sunlight have compensatory capability of diverting assimilates from root to leaf, the increased relative amount of leaf tending to compensate for reduction in light intensity. He also found that in the species showing suppression of growth by lowered light intensity there is little or no diversion of assimilates to compensate for lower energy increment. This communication presents some data on growth analysis of Indian plants to point out deviations from this general statement of Whitehead, and variations in adaptive mechanisms of the plants to light factor.

The usual growth analytical technique employing periodical harvests of plants grown under different light intensities was followed and the details of the experimental procedure have been described elsewhere<sup>6,7</sup>. The studies have shown that based on overall growth, various plants can be grouped into two categories: (a) Species like *Amaranthus spinosus*, *Cassia tora*, *Anagallis arvensis* and *Chenopodium album* show maximum growth at light intensity slightly lower than full sunlight (70–80% sunlight), and (b) species like *Eleusine indica*<sup>8</sup> and *Tribulus terrestris*<sup>9</sup> grow best only at full sunlight and any decrease in light intensity affects the growth adversely. The partitioning of the photosynthate into shoot and root reveals further interesting facts (Fig. 1).

In *Cassia tora* and *Anagallis arvensis*, the S/R ratio increases regularly with decrease in light intensity. The two species however differ from each other inasmuch as that the maximum leaf area per plant is attained in full daylight in *A. arvensis* but at 70% sunlight in *C. tora*. It is thereby quite clear that the two species adapt to decreasing light levels by expanding their shoot system at the cost of the roots. In *C. tora* increase in leaf area at 70% sunlight appears to compensate for light intensity while in *A. arvensis* it is mainly due to higher net assimilation rate (NAR = ULR, Unit Leaf Rate).

In *Amaranthus spinosus* which has been studied in some details<sup>7</sup>, the plants grown at 80% sunlight show a considerable rise in S/R ratio but a further decrease in light intensity to 57% brings down this ratio (though still greater than that at full sunlight). When the plants are subjected to still lower light levels (30%) they are unable to cope with the situation and the S/R ratio becomes smaller. It may be called the limitation of the adaptive capacity of the species or explained by assuming a minimum investment by the plant on the root system which is as important to it as the photosynthetic organs<sup>7</sup>. The studies also reveal that the plant adapts to the slight decrease in light level (to 80%) by an increase in the specific leaf area (SLA) and ULR, and only very little by contributing more photosynthates to leaf development.

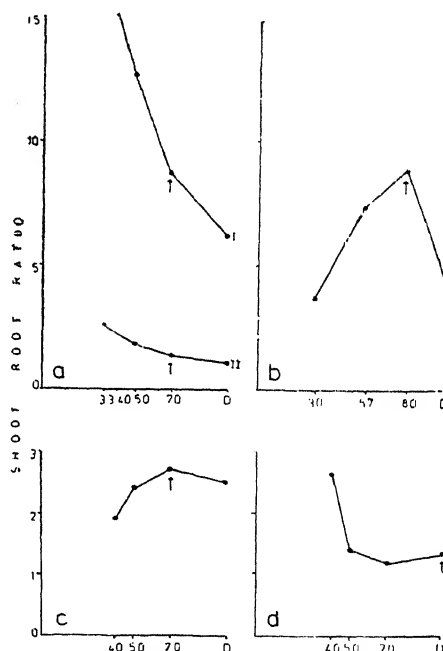


FIG. 1. Effect of different light intensities on shoot/root ratio. a—(i) *Cassia tora*, (ii) *Anagallis arvensis*, b—*Amaranthus spinosus*, c—*Chenopodium album* and d—*Eleusine indica*. The arrow indicates the light intensity at which maximum growth occurs. D = full daylight.

The photosynthates are diverted from the roots to the shoot to be utilised in growth of stem and reproductive organs. On further decrease in the light intensity, the ULR falls down appreciably while the SLA changes only slightly, and the plant diverts more photosynthate to the leaves at the expense of both roots and stems. At very low light level (30%) the ULR is at its lowest and

little rise in SLA tries to compensate for the light intensity.

In *Chenopodium album*, the S/R ratio remains more or less same between full sunlight and 50% sunlight but falls sharply thereafter. In *E. indica* as also *Tribulus terrestris*<sup>9</sup>, the situation is just the reverse. The S/R ratio shows little change from full sunlight to 50% sunlight but rises steeply at 40% sunlight.

These observations lead to the following conclusions: At lowered light intensities the plants may adapt through diversion of assimilates to the shoot but this diversion may be limited only upto certain light levels as in *Amaranthus spinosus*. In those plants which exhibit best growth in full sunlight and ordinarily do not show diversion of assimilates to shoots at low light levels, the assimilates may still be diverted at very low light intensities though without any effect on total growth. It is interesting to note that such plants show a change in habit from prostrate to erect (*Eleusine indica*<sup>8</sup>, *Tribulus terrestris*<sup>9</sup>, *Portulaca oleracea*<sup>10</sup>) at low light intensities. Thus it seems that the assimilates that are diverted to shoots are utilised in building up of more mechanical tissue to keep the plants erect.

The adaptive mechanism in the plants further involves either an increase in ULR or SLA or both. Both the ULR and SLA are independent of the capability of the plant to divert photosynthates to the shoot for build up of more leaf area (LAR). The same plant may show increase in ULR at one light level and an increase in SLA at the other. This has been amply shown by *A. spinosus*.

It is concluded that various plant species are equipped with different adaptive mechanisms to light intensity factor and of these the light regulated distribution of photosynthates to various plant parts is most important. Besides this, the changes in SLA and ULR also play important role in light adaptation.

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#### A NOTE ON FIXATION AND PRESERVATION OF CALCAREOUS ZOOPLANKTON

PROPER preservation of Zooplankton with calcareous tests and shells (Foraminifera, Radiolaria, Atlantidae, meroplanktonic gastropoda, Thecosomata, etc.), is of prime importance since studies on taxonomy and morphometry are to be based only on the morphological characters of their shells. A simple way of preservation for this purpose is to keep them as dry specimens in tubes lined with cotton. But, for anatomical and histological studies, necessitating intact preservation of soft parts, other methods are necessary. Due to high cost and great evaporation rate, the conventional preservation in ethanol was replaced by formaldehyde. The problem in formaldehyde preservation and storage is the dissolution and breakdown of the shells over a wide range of pH in varying ambient temperatures. Shelled organisms preserved in formaldehyde show varying stages of deterioration (Balachandran, 1973). Hence it was needed to evolve a formula for a suitable and cheap preservative.

About 200 formulae were tried at Indian Ocean Biological Centre in addition to 45 formulae evolved by SCOR/UNESCO/WG 23 for preservation of calcareous plankton. Formaldehyde, Dowicil 100, Ethylene glycol and phenoxetol in varying concentrations diluted with distilled water, tap water and sea water were used for these experiments. Borax, Hexamine, Calcium carbonate, Sodium acetate, Sodium ascorbate and Potassium oxalate in concentrations of 0.5 to 15% were used as neutralising agents and additives. After proper fixation of fresh plankton in 2% formaldehyde based on the results of the earlier experiments (Balachandran, 1973) shelled taxa were sorted out, transferred to the various preservatives and their conditions were periodically observed. The nature of breakdown, degree of dissolution, fragility to applied pressure and transparent and translucent state of shelled

forms and the change in PH and formaldehyde content of the preservatives were recorded.

The results show that 2% formaldehyde in distilled water formalin neutralised with excess Sodium tetraborate—( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$ ) with 3% to 5% Potassium oxalate is the best preservative. Sea water is not used for dilution since on addition of potassium oxalate, it precipitates Calcium oxalate. Distilled water rinse is therefore preferable. The Potassium oxalate combines with Calcium carbonate of shells forming insoluble Calcium oxalate, thus preventing their dissolution. 2% formaldehyde in sea water with Calcium carbonate added to saturation is found to be another satisfactory preservative. The excess Calcium carbonate in the preservative can neutralise the acidity and also saturate it to prevent dissolution of calcit in shells. Neutralised formaldehyde is added to specimen tubes to avoid excess Calcium carbonate settling on shells. For maintaining a pH between 7.0 and 7.5, it is necessary to change the preservative once in 6 months. At low pH, owing to its acidity, Calcium carbonate in the shells tend to dissolve. At higher pH (above 8.0) calcareous plankton disintegrate because of the swelling and gelatinisation of protein binding the calcareous salts. Sea water, close to saturation in its calcium content and acting as a buffer, has less dissolution rate. This may explain why huge deposits of shells lie at the bottom of sea undissolved. Brittle nature of the shells is best prevented by the addition of a few drops of glycerine into the preservative.

The author wishes to express his sincere thanks to Dr. N. K. Panikkar, former Director, National Institute of Oceanography, for giving him this unique opportunity of working in collaboration with UNESCO/SCOR/WG 23. He sincerely thanks Dr. T. S. S. Rao for going through the manuscript and for comments.

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Cochin-18, Kerala, September 13, 1973.

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#### ON METHODS OF COLLECTION, HANDLING AND STORAGE OF ZOOPLANKTON IN TROPICS

INVESTIGATIONS undertaken to locate the factors causing damage and deterioration in zooplankton samples under tropical conditions (Balachandran, 1973) suggest the use of the following improved

field and laboratory techniques for their better maintenance.

The plankton net with mesh size ranging from  $150 \mu$  to  $500 \mu$  is found to be the best as the larger mesh size of the gear causes damage to organisms due to increased flow of water, and the smaller size, due to clogging. The condition of the catch may be improved by lowering the speed of tow, by reducing the mouth area of the net and by increasing the area of the gauze. Hauls should be of short duration as long hauls which collect large amount of plankton may cause damage to the catch. The closed plankton buckets are preferable to the one with a side window or a bottom filter in order to prevent frictional damage. The practice of hosing down the sides of the net with a strong jet of water must be discontinued to prevent rupture or loss of appendages to zooplankton. During transfer of plankton from the bucket to the fixative, exposure to air must be avoided to prevent formation of artefacts. The fixation has to be carried out without delay to prevent lysis and bacterial growth. The volume of plankton to that of fixative should be in the ratio 1 : 9. Plankton must be preserved in previously numbered plastic bottles or translucent, strong, relatively unbreakable and if possible impermeable styrene jars having phenolic cap with plastic coated liners with suitable labels. Against the sample number all relevant data shall be entered in the log book. It is advisable to fill the containers completely to avoid sloshing of organisms. Separate hauls must be made for different purposes rather than splitting the same sample. Plankton samples for biochemical studies and biomass estimations are best preserved by freeze-drying. For minimal mechanical damage of the organisms, lengthy cruises must be avoided. During transport to the laboratory and on board the ship, detention of samples at improperly ventilated, warm and humid custom warehouses and on the deck for lengthy periods are best avoided.

When subsampling is inevitable, tap water should not be used without sufficient preservative so as to prevent initiation of bacterial activity and osmotic damage. Measurement of biomass by the method of displacement volume is not advisable since, during this process, the removal of interstitial fluids by shaking, blotting, filtration, etc., are found to cause damage to zooplankton. Use of plankton fractionators and divers for subsampling may be kept to the minimum. During sorting, exposure to air must be considerably reduced. In tropics, as the laboratory temperature rises upto  $32^\circ \text{C}$ , formaldehyde evaporates causing irritation and unpleasant vapours. This can be substituted with 0.5%

phenoxetol in distilled water as a good sorting medium (Balachandran, 1973) and for sorting, special types of brushes, needles, forceps and fillers have to be used. Specimen tubes having screw caps with plastic coated liners are preferable for storing the sorted specimens. The common practice of immersing tubes of specimens in a jar of preservative has to be discouraged. The volume of plankton to that of storage fluid has to be in the ratio of 1:5 and the containers should be selected accordingly. The concentration and types of additives and the diluents used in the preparation of storage fluids shall depend on the nature of plankton stored. As polythene is permeable to air, glass containers are preferred. Use of rubber washers and liners must be avoided as they melt and swell in due course. Container lids should be rust proof and air tight and must be filled to the brim to avoid air bubbles and drying up of plankton sticking to the sides. Periodic topping up after checking pH can add to improved preservation. Change of preservatives occasionally can be of additional benefit. Specimen jars properly labelled and catalogued are best stored in air-conditioned rooms and preferably in darkness, to avoid damage caused by light and temperature.

The author wishes to express his sincere thanks to Dr. N. K. Panikkar, former Director, National Institute of Oceanography, for giving him the unique opportunity of working in collaboration with UNESCO/SCOR/WG-23. He sincerely thanks Dr. T. S. S. Rao for going through the manuscript and for comments.

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#### A PRELIMINARY NOTE ON THE OCCURRENCE OF CEPHALINE GREGARINES (PROTOZOA : SPOROZOA) IN INSECTS OF KALYANI, WEST BENGAL

The cephaline gregarines have been the object of intensive study in many parts of the world since Watson<sup>1</sup> and Kamm<sup>2</sup> published their monographs on these parasites. A new classification was proposed by Grassé<sup>3</sup> who also recorded the then known

species of the group. In India, Ray<sup>4</sup> and Ray and Chakravarty<sup>5</sup> first started work on this group of protozoa and more accounts have been published from time to time by other authors. Recently we have undertaken a comprehensive survey work on the occurrence of cephaline gregarines (Protozoa : Sporozoa) in insects in and around Kalyani, West Bengal, India, and also to study the morphology, life-history and bionomics of this group of parasites. The present communication records our survey work on three orders of insects comprising 11 families and 26 species (including one nymphal stage, which could not be identified beyond family level). A brief note on some of our earlier work on the same line has already been published<sup>6</sup>.

For studying the presence of the gregarines, the host insects are collected from fields and gardens in and around Kalyani and brought to the laboratory alive, their gut contents smeared, fixed in Schaudinn's fixative and Bouin's fluid, and subsequently stained in Heidenhain's iron alum-haematoxylin. The entire mid-gut of the parasitized insects is fixed in Bouin's fluid, cut into 5 microns thick sections and stained as above for observing the intra-cellular stages of the parasites. Cysts collected from mid- and hind-gut of insects are kept in moist chambers for development of spores in living condition.

The present work was initiated on February 12, 1973, and our observations upto December 20, 1973 have been recorded in this paper. The orders, families and species to which the host insects belong, the number of specimens examined as well as infected and the percentage of infection have been indicated in Table I. It is noted that while all the six species of the order Orthoptera are infected, none belonging to the order Hemiptera is parasitized, whereas out of the 17 species of coleopteran insects more than 50% carry protozoan parasites of the group. As regards seasonal intensity, infection is very scanty during summer, increases greatly during monsoon and decreases gradually with the fall of temperature during winter.

So far, we have recorded a new genus *Phleobum* with the type-species *P. gigantum*<sup>7</sup> and a new species *Quadruspinospora chakravartyei*<sup>8</sup>, both cephaline gregarines, from *Phleoba antennata* Brunn. and *Spathosternum* sp. respectively. Preliminary studies show that parasites obtained from other insects belong to the genera *Hyalospora* Chakravarty, 1935, *Gregarina* Dufor, 1828, *Stenophora* Labbé, 1899, *Stylocephalus* Ellis, 1912 and *Quadruspinospora* Sarkar and Chakravarty, 1969, and are likely to be new species. Detailed study of their life-histories, intra-cellular development and sporulation is now being worked out.



TABLE I

Showing the orders, families and species of insects examined for cephaline gregarines and also the incidence of parasites in them

Code No.	Order	Family	Species	Number of specimens examined	Number infected	Percentage of infection
A <sub>5</sub>	Orthoptera	Acrididae	<i>Acrida exaltata</i> (Walk.)	32	23	71.8%
A <sub>10</sub>	"	"	<i>Trilophidia annulata</i> (Thunb.)	16	4	25.0%
A <sub>9</sub>	"	Pyrgomorphidae	<i>Atractomorpha crenulata</i> (Fabr.)	39	24	61.5%
A <sub>4</sub>	"	Acrididae	<i>Phleoba antennata</i> Brunn.	38	22	57.9%
A <sub>16</sub>	"	"	<i>Spathosternum</i> sp.	36	31	86.1%
A <sub>11</sub>	"	Gryllotalpidae	<i>Gryllotalpa</i> sp.	7	1	7.1%
B <sub>3</sub>	Hemiptera	Reduviidae	<i>Rhinocoris fuscipes</i> (Fabr.)	1	×	—
B	"	Lygaeidae	<i>Lygaeus hsopes</i> (Fabr.)	3	×	—
B <sub>2</sub>	"	Pyrrhocoridae	Nymph (identified upto family)	4	×	—
G <sub>1</sub>	Coleoptera	Scarabaeidae	<i>Adoretus</i> sp.	7	×	—
G <sub>2</sub>	"	"	<i>Anomala</i> sp.	2	×	—
G <sub>16</sub>	"	"	<i>Schizonycha</i> sp.	5	×	—
H	"	"	<i>Onthophagus</i> sp.	22	×	—
F	"	Tenebrionidae	<i>Gonocephalum</i> sp.	130	78	60.0%
G <sub>3</sub>	"	Chrysomelidae	<i>Raphidopalpa</i> (= <i>Aulacophora</i> ) <i>foveicollis</i> (Lucas)	98	77	78.5%
G <sub>4</sub>	"	"	<i>Haltica</i> sp.	34	3	8.8%
G <sub>5</sub>	"	"	<i>Aulacophora intermedia</i> Jacoby	98	51	52.0%
G <sub>7</sub>	"	"	<i>Lema</i> sp. <sub>1</sub>	87	29	33.3%
G <sub>8</sub>	"	"	<i>Lema</i> sp. <sub>2</sub>	19	8	41.1%
G <sub>10</sub>	"	"	<i>Oides bipunctata</i> (Fab.)	7	×	—
G <sub>13</sub>	"	"	<i>Aethomorpha</i> sp.	12	×	—
F <sub>4</sub>	"	"	<i>Corynodes</i> sp.	1	×	—
F <sub>2</sub>	"	Curculionidae	<i>Myloccerus</i> sp.	37	32	86.4%
F <sub>1</sub>	"	"	<i>Xanthoprochilus</i> sp.	19	16	84.2%
F <sub>3</sub>	"	"	<i>Lepropus</i> sp.	24	5	20.8%
E <sub>3</sub>	"	Cicindelidae	<i>Cicindela sexpunctata</i> Fabr.	2	×	—

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#### INTER-RELATIONSHIP BETWEEN THE APHID *APHIS GOSSYPYII* AND THE LEAFHOPPER. *AMRASCA DEVASTANS* POPULATIONS ON BHENDI

THE natural environment is an unstable system and as the number of insects present is ever fluctuating, the inter-relationship amongst the constituent species is complex. Differences in the abundance of one species may be expected to influence the populations of other species, when several more or less closely related pests like scales, aphids, white flies, mealy bugs, thrips and mites live and feed in the same tissues of plants as observed by DeBach<sup>1</sup>. Competition between mites, *Metatetranychus ulmi* and *Tetranychus telarius* on apple and between leafhoppers, *Empoasca* spp. and white flies, *Bemisia tabaci* G. and between leafhoppers *Erythroneura* spp. and tingide, *Corythucha ciliata* Say on Scycamore<sup>2</sup> has been reported. Jayaraj<sup>3</sup> observed that the population of *Amrasca* (*Empoasca*) *devastans* (Dist.) was low on bhendi (*Abelmoschus esculentus* L.) plants infested with *Aphis gossypii* Glover, however data were not collected. Hence the present study was made to find out the existence of such relationship between aphids and leafhoppers.

The pest population data were gathered on 'Pusa Sawani' bhendi on four leaves in each of 6 randomly selected plants in each of three replications. The counts were taken at weekly intervals commencing two weeks after sowing till a week

before pulling out of plants. As the pest population was very low at early stage and started declining as the crop matured, the counts taken from 5th to 10th weeks alone were taken for working out the correlation coefficient between aphids and leafhopper nymphs and adults.

The leafhopper nymphs were found to be less on leaves with increased numbers of aphid as evidenced from the significant correlation coefficient value ( $r = -0.619$ ) at  $P = 0.01$  level (Fig. 1). The 'r' value obtained for aphid and leafhopper adult populations was not significant (Table I).

TABLE I  
Inter-relationship between aphid and leafhopper  
populations (No. of insects/24 leaves)

Age of plants in weeks	Reproduction	Aphid Nymphs and adults	Leafhopper		
			Nymphs	Adults	Total
5	1	475	10	15	25
	2	1225	10	14	24
	3	360	9	9	18
6	1	630	15	17	32
	2	130	19	12	31
	3	435	12	8	20
7	1	380	25	11	36
	2	125	26	18	44
	3	135	24	12	36
8	1	295	33	25	58
	2	343	41	15	56
9	1	498	37	17	54
	2	129	38	25	63
10	3	97	50	18	68
	1	67	42	14	56
	2	30	57	12	69
	3	18	44	10	54
Correlation coefficient to the population aphid (n = 17)		** - 0.619	N.S.	* - 0.572	
			-0.081		

N.S. — Not Significant  
\* — Significant at  $P = 0.05$   
\*\* — Significant at  $P = 0.01$

Aphids have high reproductive potential, being parthenogenetically viviparous and apterous adults will continuously larviposit till the leaves or plants become unable to sustain the crowding, whereas

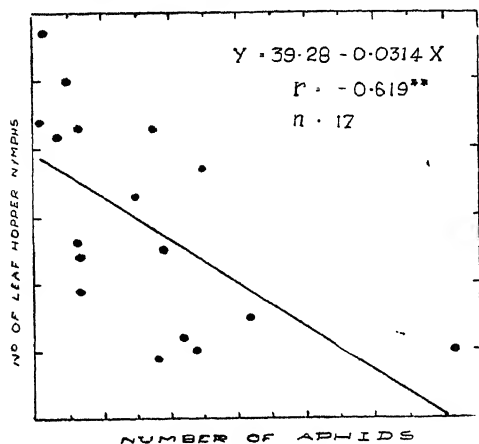


FIG. 1. Relationship between aphid and leafhopper populations.

the greater migratory tendency of leafhoppers compared to aphids might help them to get a better substrate than that already having a crowded population.

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#### A NOTE ON THE CHARACTERIZATION OF OOCYTES IN THREE TREMATODES OF *RANA TIGRINA*

IN the majority of the digenetic trematodes the oocytes pass into the oviduct before the completion of the first meiotic prophase. During its transit into the uterus the rest of the maturation processes are completed, fertilization takes place and a shell is formed around it. In the cytoplasm of these oocytes a variety of cytoplasmic bodies, which are of nutritive importance according to Yosufzai (1953) were first reported by Pennypacker (1940). Later, many workers like Markell (1943), Jone *et al.* (1946) and Willmolt (1950), etc., observed these granules in the trematodes *Probolotrema californicus*, *Macrovestibulum kepneri*, *Gigantocotyle bathocotyle* respectively. Gresson (1964), while discussing the oogenesis in trematodes, reviewed the work done

on such cytoplasmic bodies. Hanumanth Rao and Madhavi (1966) classified the oocytes into category I, II and III on the basis of the presence or absence of such granules and their nature, if present. Further, they added, "to know whether such characterization has universal applicability extensive work in these lines is called for". As a response to this, the author examined the oocytes of *Tremorchis ranarum* Mehra and Negi (1926), *Ganeo tigrinum* Mehra and Negi (1928) and *Mehraorchis ranarum* Srivastava (1934), which parasitize the common Indian frog *Rana tigrina*.

Two categories of the three mentioned by Hanumanth Rao and Madhavi (1966) are noticed in the present three parasites.

Category I exists in *T. ranarum*. The cytoplasm of the oocytes is free of cytoplasmic bodies and it presents a homogeneous appearance. The nucleus in the centre of the oocyte or, to a side, is very distinct and the cytoplasm is positive to PAS, Best's carmine and Baker's acid haematin, indicating the presence of carbohydrates especially glycogen and phospholipids.

Category III exists in *G. tigrinum* and *M. ranarum*. The oocytes contain a prominent nucleus placed either centrally or to a side. The cytoplasm consists of uniformly distributed granules, which are positive to PAS, Best's carmine and Baker's acid haematin.

The presence of category I oocytes in *T. ranarum* adds one more family, viz., *Plagiorchidae* to this category. The category III occurs both in *G. tigrinum* and *M. ranarum*. It is interesting to note that both these parasites belong to the family *Lecithodendriidae* and both of them contain the same type of oocytes. These granules as mentioned by Yosufzai (1953 a) may be the extrusions of the nuclear and nucleolar materials, which supply nutritive materials to the cytoplasm of the oocytes.

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NOTES ON A PREDATOR OF *DYSDERCUS KOENIGII* FABR. (HEMIPTERA: PYRRHOCORIDAE)\*

ABSTRACT

The pyrrhocorid bug was observed as a predator of cotton stainer *Dysdercus koenigii* Fabr. in the field. The adults of predator bug were observed to attack only on nymphs and adults of cotton stainer.

BIONOMICS of the cotton stainer *Dysdercus koenigii* Fabr. was described by Kamble (1971). A lygaeid bug *Antiloclus coqueberti* F. was observed preying on the cotton stainer *D. cingulatus* Fabr. (Ghosh, 1928; Pradhan and Menon, 1944). Lefroy (1909) reported a reduviid bug *Harpactor costalis* Rent. preying upon *D. cingulatus*. While studying the bionomics of *D. koenigii*, the author observed adults of a pyrrhocorid bug feeding on nymphs and adults of the cotton stainer in the field. The external morphological characteristics of the pyrrhocorid bug described here are based on adult specimens.

The adult is orange red in color. The head is triangular in shape and is depressed anteriorly. The labium is four-segmented; the first is thicker and longer than the remaining three segments. The fourth segment is black in color and sharply pointed. Antennae are four-segmented, filiform, and dark brown in color. The compound eyes are black in color and are quite prominent. The pronotum is trapezoid in shape and orange red in color. The coxa, trochanter, and femur of the legs are deep red; the femur is swollen and tapers at the distal end. The tibia is the longest of all segments of the legs and is black in color. All tarsi are three-segmented. The apex of fore-wing is black in color while the remaining portion is orange red. When

the wings are in repose, they cross over one another, the right wing always covering the left. On the ventral side of the abdomen, the intersegmental area appears as a black band; six bands were clearly seen. The adult closely resembles its prey and in the field, it was often difficult to recognize it from a distance. Studies on identification and biology of this predator are needed.

I wish to thank Mr. S. R. Bagal, my Supervisor and Professor, of the Entomology Department, College of Agriculture, Nagpur University, Nagpur, India, for his valuable guidance.

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CHLOROPHYLL STABILITY INDEX (CSI) OF CERTAIN ALGAE

CHLOROPHYLL stability index (CSI) is a measure of the extent the chlorophyll pigments undergo decomposition at higher temperatures. It is obtained by determining the difference between the chlorophyll value of a fresh material and that of the same material but heated to 65°C for 1 hr. Lower the value of CSI, greater is the stability of chlorophyll at higher temperatures. In several instances the CSI has been correlated with drought resistance of certain crop plants<sup>1</sup>.

In recent years much attention is paid to the exploitation of algae for the production of animal feed substitutes and/or in conserving the nitrogen status of cultivated soils<sup>2-4</sup>. A continuous process of solar energy conversion into organic matter can be maintained with algae which are efficient photosynthesizers. Nevertheless, these green or blue-green algae have ability to adapt themselves to varying environmental conditions, high temperatures, light intensities and pH have striking influence

on the metabolism of algae<sup>5</sup>. The isolation of a high temperature strain of *Chlorella* by Sorokin and Myers is a landmark in this direction<sup>6</sup>. Temperatures beyond 45°C have been observed to inactivate the photosynthetic activity in *Nostoc* and *Chlorella*<sup>7</sup>. In the present study the chlorophyll stability indices of a few strains and species of algae have been reported.

Murthy and Majumder<sup>1</sup>. The final volume of the extract was made up to 40 ml with methanol : acetone extractant. The chlorophyll content in the sample was read in a Klett Summerson photoelectric colorimeter using a red filter. Similar readings were taken from unheated samples. The difference between the two readings gave the Chlorophyll Stability Index. The results are presented in Table I.

TABLE I  
*Chlorophyll stability Index of certain algae\**

Algae species	Colorimeter reading		Chlorophyll Stability Index (CSI)	Standard Error
	Unheated sample	Heated sample		
1. <i>Chlorella vulgaris</i> Beijerinck (Japanese strain)	266	254	12	7.20
2. <i>Chlorella vulgaris</i> Beijerinck (Local strain)	176	144	32	9.85
3. <i>Chlorella vulgaris</i> Beijerinck (Sewage Isolate—I)	184	165	19	3.71
4. <i>Chlorella vulgaris</i> Beijerinck (Sewage Isolate—II)	79	55	24	6.25
5. <i>Chlorella vulgaris</i> Beijerinck (California strain)	270	232	38	5.29
6. <i>Chlorella pyrenoidosa</i> Chick (Arizona strain)	190	168	22	3.46
7. <i>Scenedesmus obliquus</i> (Turpsin) Kuetz.	230	188	42	11.02
8. <i>Anacystis nidulans</i> (Richt) Drouvet and Dailey	155	111	44	4.05

\* Data represent average of three estimations.

The different algal cultures used in the present study were obtained from the culture collections of the Microbiology Laboratory, Tamil Nadu Agricultural University. The algae were maintained in Myer's agar medium<sup>8</sup>. One loopful of the culture was transferred to 500 ml of liquid medium in 1000 ml Erlenmeyer flasks. The flasks were incubated at room temperature (28°C) for 20 days in the diffused sunlight (1075 lux), when the algae attained maximum growth. The cells were harvested by centrifugation at  $2,100 \times G$  for 10 min. Excess moisture in the algal mass was removed by blotting them in folds of country filter-paper. One g of the fresh-material was suspended in 20 ml of distilled water in test-tubes and heated on a water-bath at 65°C for one hr. Using methanol : acetone (1:1 v/v) the chlorophyll was extracted following the procedures of

*Chlorella vulgaris* Beijerinck, Japanese strain, had the least value of CSI among the various strains tested, and *Anacystis nidulans* the maximum value. As lower the value greater the chlorophyll stability, it follows that the Japanese strain of *Chlorella* possesses maximum quantity of chlorophyll as against the local strain (Sewage isolate. II) containing the least amount.

This is of primary concern because during summer months the temperature rises to higher limits to affect the survival of *Chlorella*. As suggested by Nakamura<sup>2</sup>, in places where large scale multiplication of *Chlorella* is contemplated this temperature effect could be avoided by (1) addition of cold water to the culture pond, (2) repeated stirring, (3) by setting up temporary awning; this will prevent high temperatures and excess sunlight which itself has been found to be harmful leading

to bleaching of the cells. From the results of the present study, it is suggested that *C. vulgaris*, Japanese strain, may withstand high temperatures and may be recommended for large-scale cultivation.

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#### CYTOLOGICAL STUDIES IN *CHLOROPHYTUM BHARUCHAE* ANSARI, RAGHAVAN ET HEMADRI

The genus *Chlorophytum* Ker-Gawl. (Liliaceae) is represented by about 50 species in the tropical and subtropical countries of the world. Out of these a little more than a dozen species occur in the Indian subcontinent. *C. bharuchae* came to be known recently. It occurs in the Western India and some parts of the Southern and Central India. It grows well in and around Aurangabad on slopes of hills. It can be distinguished from other related species by its glaucous, rather undulate leaves, occasionally branched lax raceme, perianth segments marked with a brown line on the back and relatively small anthers. The root fibres are thick, fleshy and thus perennial as in many other species.

This species, however, can be easily mistaken for *C. glaucum* Dalz. with which it very closely

resembles but the latter has much restricted distribution, being known only from the higher altitudes in the Western Ghats and having a dense and often unbranched raceme.

The cytology, particularly the karyomorphology, has been worked out in respect of about 16 species from different parts of the world and it is a well known fact that the genus has two basic numbers, viz.,  $X=7$  & 8. The species studied so far are known to be at diploid, tetraploid as well as polyploid levels and some probably with amphidiploid composition. The cytological studies in the genus have been recently reviewed by Sheriff and Chennaveeraiah (1972).

The living specimens of *C. bharuchae* were collected from the hillslopes along the outskirts of Aurangabad town and from near about areas. The root tips were pretreated with 0.01% colchicine for about three hours and these and flower buds were fixed in 1:3 acetic-alcohol. Acetoorcein (1%) was used for staining.

*C. bharuchae* is a diploid with  $2n=16$ . It has one pair of metacentric, two pairs of submetacentric and the remaining five pairs of acrocentric chromosomes. The karyotype is more or less symmetrical and its absolute length is  $15.4 \mu$ .

Microsporogenesis appears to be normal. The meiocytes show eight clear bivalents at meiotic metaphase I. One, or in some instances, two nucleolii were observed at diakinesis. Pollen grains are two nucleate at the time of anthesis. A sufficiently high percentage of their viability is indicated by the regular meiotic behavior together with rich stainability.

A large number of capsules are produced per plant but the development of viable seeds is surprisingly very low. The plants appear to be propagating mainly by means of the perennial rootstocks.

My grateful thanks are due to Prof. K. B. Deshpande of this department for providing facilities.

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## SHORT SCIENTIFIC NOTES

### Ear Blight and Scab of Wheat in Arunachal Pradesh

Samples of wheat (Sonalika, Kalyan Sona, Safed Lerma, Lerma Rojo) infected by *Fusarium avenaceum* (Fr.) Sacc. causing 'ear blight' were received from Jomlo seed farm of Along, Siang District (Arunachal Pradesh) in February, 1970. Subsequently this was also recorded on samples received from Gauhati (Assam). Colony on PDA, white with rosy tinge and profuse aerial growth. Conidia 3-5 (-6), mostly 5 septate, curved but middle portion almost straight and gradually pointed at the ends, (20 —) 25-44  $\times$  3° 3-5  $\mu$ .

In April, 1973, another sample of wheat earheads (Kalyan Sona) was received from the same localities of Arunachal Pradesh including Kabu and found to be affected by a different malady, 'scab' caused by *Gibberella zeae* (Schw.) Petch. Innumerable black, gregarious, occasionally solitary perithecia were found on the glumes. Conidia were also observed in a pinkish mass. The first symptom appeared as drying up of only 3 to 4 spikelets which spread to the entire earhead within a month.

Individual perithecia: bluish, sometimes with violet tinge, ostiolate, may or may not be shortly beaked, ovoid, 124-174 (diameter)  $\times$  180-250 (height)  $\mu$ ; asci clavate, short pedicellate, 58-83  $\times$  10-13.3  $\mu$ ; ascospores arranged irregularly biserially, hyaline, fusiform with rounded ends, straight or dorsoventral, 3 septate, constricted at septa, 18.3-25  $\times$  3.7-4.6  $\mu$ . Conidia like above and measure (27 —) 40-60  $\times$  4.2-5.8  $\mu$ .

*F. avenaceum* causes foot rot, seedling blight and ear blight of wheat in Europe, U.S.A., Canada, Australia and New Zealand and *G. zeae* scab in Europe, U.S.A. and Canada<sup>1</sup> but this seems to be the first record for both these diseases in India. However, *F. avenaceum* has been isolated from soil in Madras<sup>2</sup> and *G. zeae* as *G. saubinentii* (Mont.) Sacc. recorded on earheads of rye in Shillong<sup>3</sup>.

As the diseases are favoured by a temperature ranging from 10° C and above with wet environment<sup>1</sup>, weather at Along which was found near to these conditions (R.H. 84.4 to 93.4%, rainfall 42.4 to 86.8 mm. Min. temp. 11.1 to 13.5° C and Max. temp. 20.1 to 24.1° C) during January-March seem to be responsible for heavy occurrence of scab in 1973.

My thanks are due to the District Agricultural Officer, Along, for sending the specimens.

Assam Agricultural Univ.,

A. K. Roy.

Jorhat-785013,

Assam, September 1, 1973.

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2. — and Bisby, G. R., *The Fungi of India* (Revd. by R. S. Vasudeva), I.C.A.R., New Delhi, 1960.
3. Chowdhury, S., *Curr. Sci.*, 1947, 16, 152.

### Computer Program for Estimating Stability Parameters in Crop Plants

Evaluation of crop varieties for their suitability for general cultivation under varying ecological and agroclimatic systems, is of prime concern to plant breeders in their breeding work. The stability of the newly synthesised superior performing genotypes are tested by exposing them to varying environmental stresses over years and locations. A number of statistical parameters have been used in the past to measure this inherent potentiality of the cultivars to withstand these environmental rigours and stresses.

#### A statistical model

$$y_{ij} = \mu v_i + \beta_i I_j + \delta_{ij}$$

proposed by Eberhart and Russell<sup>1</sup> (1966), however, is the one now being widely used. Eberhart and Russell (1966) has defined a stable variety as one with regression of individual mean yield on environmental index ( $b$ ) = 0. and deviation from regression ( $D^2$  di) = 0.

A computer program which estimates all these parameters has been developed and documented for the Indian Made TDC-12 Computer. The program is written in 4 = - K fortran language. With suitable modifications in the dimension statement, the program can handle a large number of varieties in many years and locations. Presently, it handles 25 varieties grown over 4 seasons at the Horticultural Research Centre, Patharchatta, GBPUAT, Pantnagar (U.P.).

The computer output contains, means of varieties over-all the locations, regression of individual mean yield on environmental index, deviation from regression, test of deviation from regression for

each variety and an analysis of variance table partitioning all sources of variation as described by Eberhart and Russel (1966).

A listing of the program along with a worked example could be obtained from us.

We are grateful to Dr. N. K. Anant Rao, Dean, Agriculture ; Dr. K. G. Gollakota, Dean, P.G.S., and Dr. D. D. Pant, Dean, C. B. S. H., for facilities.

The first author acknowledges Indian Council of Agricultural Research for the award of a Senior Fellowship.

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Pantnagar, Nainital, U.P.,  
December 14, 1973.

K. V. PETER.  
V. K. SRIVASTAVA  
  
B. RAI.  
R. C. JAIN.

1. Eberhart, S. A. and Russel, W. A., *Crop Sci.*, 1966, 6, 36.

## REVIEWS AND NOTICES OF BOOKS

'Discrete Models'. By Donald Greenspan, (Addison-Wesley Publishing Company, Reading, Mass.), 1973. Pp. xiv + 165. Price : Cloth : \$16.00 ; Paper \$8.50.

This book is an interesting addition to the series of monographs on Applied Mathematics and computation. It is concerned with a new type of mathematical modelling where, a discrete approach is followed which is more amenable to computer analysis than the current physical theories.

The book consists of ten chapters and some interesting appendices.

- Chapter I : Fundamentals of discrete model theory.
- Chapter II : Discrete oscillators.
- Chapter III : Nonlinear string vibrations.
- Chapter IV : Planetary motion and discrete Newtonian gravitation.
- Chapter V : The three-body problem.
- Chapter VI : The  $n$ -body problem.
- Chapter VII : Discrete fluid models.
- Chapter VIII : Symmetry in discrete mechanics.
- Chapter IX : Other forms of discrete mechanics.
- Chapter X : Discrete special relativity.
- Appendix A—The Van der Pol oscillator.
- Appendix B—A discrete Hamilton's principle.
- Appendix C—Conservation of Angular momentum in three dimensions.

An interesting set of research problems and good bibliography are provided at the end.

In conclusion, I feel that this book will be of interest to theoretical physicists, Computer Scientists and applied mathematicians. A one semester course on this subject will be of great value in Master's level Physics, Mathematics and Computer Science program.

E. V. K.

'Matrices and Linear Systems—A Programmed Introduction'. By Gaylord M. Merriman and Andrew Sterrett. (W. A. Benjamin, Inc., Menlo Park, California, U.S.A.), 1973. Pp. viii + 436.

This is an exciting and interesting book on Linear and Matrix algebra. Beginners would develop an interest to read through the book cover to cover, without a feeling of 'heaviness' which normally occurs while reading most mathematical texts. For practicing applied mathematicians and teachers this book is very convenient as a quick reference text and it exemplifies how mathematics can be taught at the university level in the most exciting and interesting way.

The book uses a programmed approach so that the reader can check his understanding, soon after the presentation of concepts. However, the organization is such that at no instance, he can feel bored.

The chapterwise organization is given below :

- Chapter 1 : Introduction to Linear Systems and Matrices.
- Chapter 2 : Matrices.
- Chapter 3 : Some Special Matrices.
- Chapter 4 : Applications of Matrices to Linear Systems.
- Chapter 5 : Inverse of a Matrix.
- Chapter 6 : Vector Spaces and Vectors I.
- Chapter 7 : Vector Spaces and Vectors II.
- Chapter 8 : Row Space and Row Rank of Matrix.
- Chapter 9 : Rank of a Matrix.
- Chapter 10 : Existence Theorems for Solutions of Linear Systems.

A reasonably good bibliography is available at the end for those who want to go deeper into other aspects.

I have given this book to some of my students at Bachelor's and Master's Engineering level and



research students in mathematics and they all have the unanimous opinion that they could learn the concepts of matrix theory and linear algebra in the sense 'At last, I finally understand what it all means'. The authors deserve congratulations for their endeavour.

I strongly recommend this book for Science, Engineering and Economics students, at University level. Libraries should hold multiple copies of this book for reference.

E. V. K.

*Serials in Microform 1973/74*. Published by Xerox University Microfilms, Ann Arbor, 300 North Zeeb Road, Michigan. 1973. Pp. xi + 683. Price \$ 4.95.

For librarians facing perpetual problem of shelving space, microform publication (microfiche, microcard, microfilm, etc.) has been of considerable relief. Publishers are now bringing out micro publications of not only back volumes of Journals but even of the current issues. In this context *Serials in Microform 1973/74* serves as a very useful reference tool to librarians.

This publication lists about 7,000 items—journals, newspapers and documents on microfilm and microfiche available from Xerox University Microfilms, Ann Arbor, Michigan. It covers such varied subjects as arts, health sciences, natural and physical sciences, social sciences and sports.

The book is divided into three sections. The main section is the alphabetical listing giving the complete details of the back volumes available and the price, current volumes and the price, whether the title is indexed in any other source, place of publication and the order number. The second section is something like a subject index and helps in locating material on the subject of interest. The subjects are arranged alphabetically. The third section describes different microform readers and storage cabinets for microfilm and microfiche.

A. R.

*Rothamsted Experimental Station—Report for 1972*. Parts I & II. (Lawes Agricultural Trust, Harpenden, U.K.), 1973. Part I, Pp. 403. Part II, Pp. 217. Price: Both Parts £2.50 (Post Free).

The Annual report for 1972 of the famous agricultural research station details further progress of work carried out in various branches of agriculture. Some of the interesting observations are the improvement in soil structure brought about by the addition of the supernatant of leaf extracts after coagulation of the leaf proteins. The role played by the Mycorrhizal fungi in phosphorus nutrition of plants has also been demonstrated.

Part II of the report contains papers from the members of the Station. These papers give extensive details about the long term trials conducted at the station. They incorporate data and information about the experiments that cannot be usually be condensed for publication in the regular journals. This year there are valuable papers on water use by crops, magnesium nutrition of plants and organic matter turn over under different cropping systems.

V. N. V.

## ANNOUNCEMENTS

### Award of Research Degrees

Osmania University, Hyderabad, has awarded the Ph.D. degree in Mathematics to Shri Sambasiva Rao; Ph.D. degree in Biochemistry to Miss Nirmala; Ph.D. degree in Botany to Shri Ch. Manohara Chary, Miss Usha and Miss K. Umabala; Ph.D. degree in Zoology to Shri A. Purushothama Rao.

### Books Received

*Text-Book Series in Agricultural Chemistry—Fundamental Principles*. By A. Sankaram. (The Bangalore Press, 88 Mysore Road, Bangalore-18). 1973. Pp. viii + 186. Price Rs. 17.50.

*Mineral Nutrition of Plants—Principles and Perspectives*. By Emanuel Epstein. (Wiley Eastern P. Ltd., Pub., J. 41, South Extension 1, New Delhi-110049), 1972. Pp. ix + 412. Price \$ 4.80.

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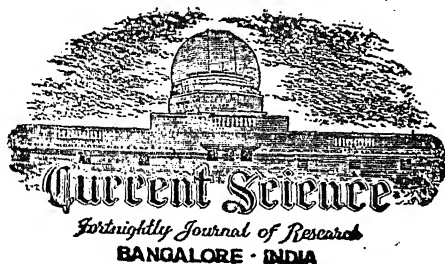
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*Institutions*: India Rs. 48.00; Foreign £5.00; \$12.00.

*Individuals*: India Rs. 24.00; Foreign £3.50; \$8.00.

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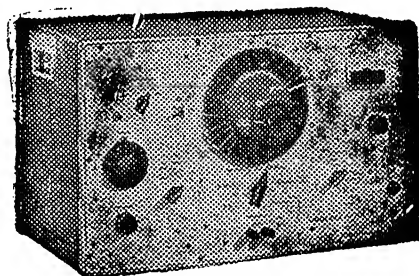
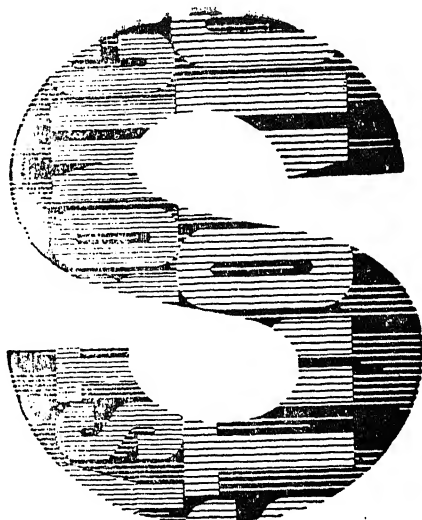
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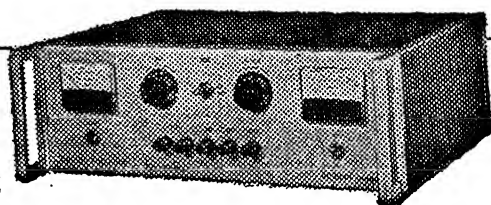
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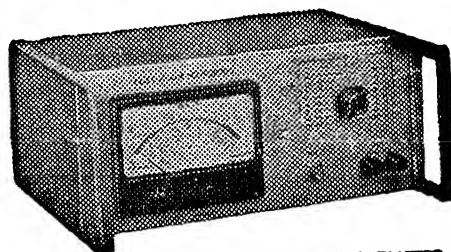
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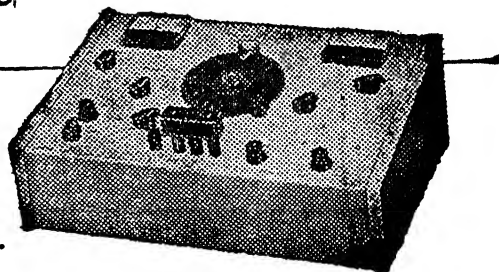
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# A FINITE NEUTRINO REST MASS FROM GENERAL RELATIVITY

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## ABSTRACT

The weak interaction having the universal Fermi coupling constant and mediated by a massive intermediate boson is related to the metric of the surrounding space-time through a line element analogous to the well-known Reissner-Nordström line element for the electromagnetic interaction. Together with a fundamental beta-decay length this predicts a finite rest mass for the neutrino which is well within experimental upper limits. The corresponding intermediate boson mass is also obtained. Further, making use of the line element for a particle with 'Yukawa charge', the pion-proton mass ratio is obtained.

## INTRODUCTION

RECENTLY it was pointed out that the appropriate line element for a mass point with 'Yukawa charge'  $g$  is given as<sup>1,2</sup>:

$$ds^2 = \left(1 - \frac{2m}{r} + \frac{\alpha' \exp[-2\mu r]}{2r^2}\right) c^2 dt^2 - \left(1 - \frac{2m}{r} + \frac{\mu \alpha' \exp[-2\mu r]}{r}\right) dr^2 - r^2 d\Omega^2, \quad (1)$$

where  $m = GM/c^2$  ( $M$  being the mass and  $G$  the Newtonian gravitational constant), and  $d\Omega^2 = r^2 d\theta^2 + \sin^2\theta d\phi^2$ . The Yukawa potential is:

$$\phi(r) = \frac{g \exp(-\mu r)}{r}. \text{ In Eq. (1), } \alpha' = Gg^2/c^4.$$

The dimensionless strong (Yukawa) interaction coupling constant (pion-nucleon coupling constant is given by:  $g^2/\hbar c = 14.8$ . In analogy with the electromagnetic coupling constant,  $\alpha \equiv e^2/\hbar c = 1/137$  ( $e$  being the electric charge), this suggests that  $g$  can be considered as some kind of charge for strong interactions or a Yukawa charge. The exponential factor in Eq. (1) takes into account the short range of the strong interactions, with  $\mu = m_\pi c/\hbar$ ,  $m_\pi$  being the pion mass, the pion mediating the interaction. In the long-range approximation, (i.e.,  $\exp[-2\mu r] \approx 1$ ), the line-element given in Eq. (1) becomes similar to the Reissner-Nordström line-element for the electromagnetic interaction. Now the weak interaction (or beta-decay interaction) is also a short-range interaction. By analogy with strong interactions we could write a corresponding Yukawa potential, given by:

$$\phi_w(r) = \frac{g_w \exp(-\mu_w r)}{r},$$

where  $g_w$  is the weak interaction 'charge' corresponding to the dimensionless coupling constant  $g_w^2/\hbar c$ ; and  $\mu_w = m_w c/\hbar$ ,  $m_w$  being the mass of the mediating intermediate boson. This enables us to write the line-element which relates

this potential to the metric of the surrounding space time as:

$$ds^2 = \left(1 - \frac{2m}{r} + \frac{a_w \exp[-2\mu_w r]}{2r^2}\right) c^2 dt^2 - \left(1 - \frac{2m}{r} + \frac{\mu_w a_w \exp[-2\mu_w r]}{r}\right) dr^2 - r^2 d\Omega^2, \quad (2)$$

where:

$$a_w \equiv \frac{Gg_w^2}{c^4}.$$

## THE FUNDAMENTAL BETA-DECAY LENGTH AND THE NEUTRINO REST MASS

The universal Fermi coupling constant for beta decay is given by

$$G_F = 1.5 \times 10^{-49} \text{ erg. cm}^3. \quad (3)$$

It is interesting that using  $G_F$  we can construct a unique length as

$$l_w = \left(\frac{G_F}{\hbar c}\right)^{\frac{1}{2}} = 6 \times 10^{-17} \text{ cm.} \quad (4)$$

We shall characterize  $l_w$  as a fundamental length for beta-decay, as it is constructed solely from the universal constants  $G_F$ ,  $\hbar$  and  $c$ . For  $g_w$  we can now write:

$$g_w^2 = G_F \left(\frac{m_e c}{\hbar}\right)^2, \quad (5)$$

$m_e$  being the electron rest mass. As we are using  $m_e$  on the right side of Eq. (5) we shall replace all subscripts  $W$  by  $W_e$ . Thus  $g_{w_e}$  is a kind of electron-neutrino 'charge'. Replacing  $m_e$  in Eq. (5) by the muon rest mass  $m_\mu$ , we get the corresponding muon-neutrino charge  $g_{w_\mu}$ . Then the dimensionless constants are  $g_{w_e}^2/\hbar c$  and  $g_{w_\mu}^2/\hbar c$ .

The current idea<sup>3,4</sup> about the weak and electromagnetic interactions is that they are not merely analogous phenomena but different aspects of a unified dynamical mechanism. Our approach in the present problem will be in conformity with this idea. We shall consider the electron-neutrino as the counterpart of the electron in weak interactions

with its characteristic weak charge  $g_{we}$ . The muon-neutrino then plays the corresponding role for the muon. Earlier we had shown<sup>1,5</sup> that the Reissner-Nordström metric exhibits an equilibrium point at  $r_0$ , given by :

$$r_0 = \frac{e^2}{m_e c^2} = 2.8 \times 10^{-13} \text{ cm.}, \quad (6)$$

(for the electron rest mass  $m_e$ ).

Now in an exactly similar manner we can show that in the long-range limit, *i.e.*,  $\exp[-2\mu_w r] \sim 1$ , the metric given by Eq. (2) has an equilibrium point at  $r_{0we}$  given by :

$$r_{0we} = \frac{g_{we}^2}{2m_\nu c^2}, \quad (7)$$

where  $m_\nu$  is the electron-neutrino rest mass (*i.e.*, we replace the mass  $M$  occurring in the metric by  $m_\nu$ ). Now in the electromagnetic case the equilibrium point occurs at the classical electromagnetic radius  $r_0$  which is a kind of 'characteristic length' for electromagnetic interactions. By analogy one would expect the equilibrium radius  $r_{0we}$  to be the characteristic beta-decay length  $l_w$  given by Eq. (4).

Thus :

$$m_\nu = \frac{g_{we}^2}{2l_w c^2}, \quad (8)$$

Substituting for  $l_w$  and  $g_{we}$  from Eqs. (4) and (5) we have :

$$m_\nu = \frac{1}{2} \left( \frac{G_F c}{\hbar^3} \right)^{\frac{1}{2}} m_c^2, \quad (9)$$

This gives  $m_\nu \simeq 0.7$  electron-volts.

This is consistent with the experimental upper limit<sup>6</sup> of  $m_{\nu_e} < 60$  eV. Similarly for the muon-neutrino we have by analogy

$$m_{\nu_\mu} = \frac{1}{2} \left( \frac{G_F c}{\hbar^3} \right)^{\frac{1}{2}} m_\mu^2, \quad (10)$$

giving  $m_{\nu_\mu} = 0.028$  MeV consistent with the experimental upper limit<sup>6</sup> of  $m_{\nu_\mu} < 1.5$  MeV. Also from the uncertainty principle, the 'range'  $r_w$  of the intermediate W boson is given by :

$$r_w = \frac{\hbar}{m_w c}, \quad m_w \text{ being the boson mass.}$$

Equating this to the characteristic length  $l_w$  gives :

$$m_w = \left( \frac{\hbar^3}{G_F c} \right)^{\frac{1}{2}} \simeq 250 \text{ BeV.} \quad (11)$$

This is to be compared with other estimates such as those of Schwinger<sup>7</sup> who obtains :

$$m_w = \left( \frac{4\pi a}{2^{3/2} G_F} \right)^{\frac{1}{2}} = 53 \text{ BeV.}$$

There is as yet no reliable experimental evidence for the existence of the W-boson although many different estimates of its mass are predicted by

various theories! The prevailing opinion now is that it should be a heavy boson (if it exists) with a mass perhaps several times the proton mass ( $\sim 1$  BeV) as there is no evidence for it with the energies available in present-day accelerators.

#### THE PION-PROTON MASS RATIO

It can easily be shown that in the long-range limit the line element given by Eq. (1) for a particle having the proton mass  $m_p$  with Yukawa 'charge'  $g_s$  has an equilibrium point  $r_{os}$  given by :

$$r_{os} = \frac{g_s^2}{2m_p c^2}. \quad (12)$$

As was done in the case of weak interactions above, we can equate this to the range (or characteristic length) of the strong interaction mediated by the pion, *i.e.*,  $\hbar/m_\pi c$ ,  $m_\pi$  being the pion rest mass.

Thus

$$\frac{g_s^2}{2m_p c^2} = \frac{\hbar}{m_\pi c}, \quad (13)$$

or

$$\frac{m_\pi}{m_p} = 2 \frac{\hbar c}{g_s^2}.$$

Using the experimental value for the strong interaction coupling constant, *i.e.*,

$$\begin{aligned} \frac{g_s^2}{\hbar c} &\approx 14.5, \\ \frac{m_\pi}{m_p} &\approx \frac{1}{7}. \end{aligned} \quad (14)$$

Using  $m_p = 938$  MeV; we have  $m_\pi = 134$  MeV, close to the observed value  $m_\pi = 139$  MeV.

#### EINSTEIN FIELD EQUATIONS WITH DIFFERENT COUPLING CONSTANTS

The Einstein field equations<sup>8</sup>

$$G_{\mu\nu} = \kappa T_{\mu\nu}, \quad (15)$$

relate a geometrical invariant quantity (*i.e.*, the Einstein tensor  $G_{\mu\nu}$ ) on the left side to an invariant physical quantity (*i.e.*, the conserved energy-momentum tensor  $T_{\mu\nu}$ ) on the right side through a proportionality constant  $\kappa$ . It must be emphasized that the derivation of Eq. (15) places no restriction whatsoever on the value of the constant  $\kappa$ . As Einstein used Eq. (15) as the basis for his relativistic theory of gravitation, he had to choose  $\kappa = -8\pi G/c^4$ ,  $G$  being the Newtonian constant of gravitation, so as to be consistent with the Newtonian gravitational theory and its related Poisson equation. Following him it has become customary to always relate  $\kappa$  to the Newtonian gravitational constant  $G$ . Almost all applications of general relativity have hitherto been in the realm of astronomy and astrophysics and hence this choice of  $\kappa$  was suitable as gravitational forces

are predominant in the macrocosmos. In principle, however, all interactions have their own energy-momentum sources and can give rise to gravitation. These interactions have their own characteristic coupling constants. We point out below some interesting physical consequences of identifying these coupling constants with  $\kappa$ , i.e., by putting different values for  $\kappa$ . From quantum geometrodynamical considerations, Wheeler<sup>9</sup> has pointed out that at distances of the order of the Planck length, i.e.,

$$\left(\frac{\hbar G}{c^3}\right)^{\frac{1}{2}} = 1.6 \times 10^{-33} \text{ cm},$$

quantum effects become important in general relativity. The uncertainty principle then shows that at such distances quantum gravitational effects proceed by exchange of quanta of masses of the order of the Planck mass.

$$\left(\frac{\hbar c}{G}\right)^{\frac{1}{2}} = 2.2 \times 10^{-5} \text{ g}$$

which in turn implies density fluctuations of the absurdly high value of  $10^{93} \text{ gm/cc}$  or  $10^{114} \text{ erg/cc}$ ! This would also be the density (it would be infinity if quantum effects were ignored) of the inevitable singularity (e.g., Penrose and Hawking<sup>10</sup>) occurring in gravitational collapse or the initial density of the singularity in big-bang cosmological models, e.g., Harrison<sup>11</sup>). This absurdly high density could be scaled down by invoking  $f$ -gravity or the strong gravity mediated by massive spin-2  $f$ -meson. This has a coupling constant  $G_f$  shown to be<sup>1,12</sup>,  $10^{38}$  times the Newtonian value  $G_N$ . With this value of  $G = G_f$ , the Planck length becomes  $\sim 10^{-14} \text{ cm}$ , the Planck mass of the order of the proton mass ( $10^{-24} \text{ g}$ ) and the quantum gravitational density fluctuations take on a much more reasonable value of  $\sim 10^{17} \text{ g/cc}$ . As most theories of superdense matter<sup>13</sup> (e.g., in neutron stars) picture the densest state to consist predominantly of hadrons, it is natural that  $f$ -gravity must be invoked to discuss quantum gravitational effects. The quantities characteristic of quantum gravitation then come much closer to values encountered in elementary particle physics. With  $G_f$  the exchange quanta will have masses of the order of the  $f$ -meson mass which is precisely what we expect. We shall now relate  $\kappa$  to the Fermi coupling constant  $G_f$ . From  $G_f$  we can construct a quantity having the same dimensions as the gravitational constant  $G$  as

$$G_w \rightarrow G_f \left(\frac{c}{\hbar}\right)^2 = 1.4 \times 10^{36}.$$

Then the characteristic Planck length

$$\left(\frac{\hbar G}{c^3}\right)^{\frac{1}{2}},$$

with  $G_w$  replacing  $G$  becomes :

$$L = \left(\frac{G_f}{\hbar c}\right)^{\frac{1}{2}},$$

which is precisely the characteristic beta-decay length  $l_w$ ! The mass of the exchange quanta then become

$$\left(\frac{\hbar c}{G_w}\right)^{\frac{1}{2}} = \left(\frac{\hbar^3}{G_f c}\right)^{\frac{1}{2}},$$

precisely the value obtained for the mass of the intermediate boson in Eq. (11). Thus  $l_w$  is the equivalent of the 'quantum gravitational length' (i.e., Planck length) in weak interactions and  $m_w$  is the corresponding quantum gravitational mass (i.e., Planck mass) for weak interactions. Similarly by using the strong interaction coupling constant we can show that the Planck length scales up to the pion Compton wavelength and the Planck mass scales down to the pion rest mass. The intermediate boson and the pion are therefore the corresponding 'quantum gravitational mass' for weak and strong interactions respectively. The virtual quanta mediating quantum gravitational interactions have their gravitational radius as the Planck length, i.e., the same as their interaction range. These virtual quanta can be likened to 'quantum black holes' as their mass is always confined within their gravitational radius. From the above we can see that the 'virtual' pions and intermediate bosons which mediate strong and weak interactions are also, similar entities with their corresponding masses and 'gravitational radii' scaled by the respective interaction coupling constants.

#### CONCLUDING REMARKS

We see that a considerable amount of physics is hidden in the Einstein field equations if new interpretation of the 'cosmological constant' and  $\kappa$  were given<sup>1</sup>. From rather elementary considerations we have been able to present a unified picture of various interactions by incorporating the coupling constants of these different interactions into the field equations and their solutions. This is consistent with recent work<sup>12</sup> of the authors where it was shown that using the coupling constant  $G_f$  for  $f$ -gravity instead of the Newtonian constant would make general relativity far more relevant to particle physics. The calculation of the neutrino rest mass given in this paper is also in conformity with another recent work<sup>14</sup> of the present authors where the masses of stable elementary particles were tried to be understood solely in terms of the interactions they undergo.

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## TEMPERATURE DEPENDENCE OF MAGNESIUM STIMULATED ADENOSINE TRIPHOSPHATASE ACTIVITY DURING AGING OF THE CENTRAL NERVOUS SYSTEM OF RAT

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### INTRODUCTION

TEMPERATURE dependence of  $Mg^{++}$  ATPase has been studied in mammalian brain homogenates<sup>1-3</sup> and microsomal preparations<sup>4</sup>. Comparisons of these homeotherms with heterothermic animals<sup>4</sup> and poikilothermic animals<sup>5</sup> have shown that ATPases in these animals are less sensitive to low temperatures. The properties of the total ATPase enzyme complex in mammalian brain preparations have been studied in detail<sup>6-9</sup> but more attention has been concentrated on the  $Na^+-K^+$  ATPases because of their relationships with the oxidative phosphorylation and active transport processes.  $Mg^{++}$  ATPase is responsible for the control of passive permeability of excitable cells<sup>10-11</sup> and probably of all cells and cellular organelles<sup>12-13</sup>. Bowler and Duncan<sup>14</sup> have suggested that  $Mg^{++}$  ATPase of the excitable cells has a role in the control and maintenance of the excitability of these cells. It is also known that the development of the receptor potential during the excitation of sense organs reflects a change in the passive permeability of the receptor membrane to cations<sup>15</sup>.

Studies on the effect of temperature on  $Mg^{++}$  ATPase activity in rat brain during postnatal development is very little, and no data is available on the temperature dependence of the enzyme activity in the aging central nervous system. This paper is a study of the temperature dependence of the magnesium stimulated ATPase activity during the aging of the central nervous system.

### MATERIAL AND METHODS

Albino rats (Wistar strain) of 1 day, 3, 13, 44 and 87 weeks age used for the study were maintained at  $28 \pm 2^\circ C$  on a commercial diet (Rat and mice feed purchased from Hindustan Lever Ltd., Bombay).

The animals were decapitated and different regions of the brain (Cerebrum, Cerebellum, Medulla and Optic lobes) excised immediately and weighed in precooled beakers of 5 ml capacity. Tissues were homogenised in glass homogenisers in ice-cold 0.13 M Tris-buffer (pH 7.4) in 0.25 M sucrose as described by Tirri *et al.*<sup>1</sup> to give a homogenate which contained, depending upon the age of the animal 14 to 20 mg of tissue per ml. This was centrifuged at 6000 r.p.m. for 30 minutes and the supernatant was used for the study of the enzyme activity.

Incubation medium contained 5 mM ATP in Tris-buffer, 5 mM  $MgCl_2$  and 0.2 ml of the enzyme extract all in a volume of 1.8 ml. The above were incubated at  $10^\circ$ ,  $20^\circ$ ,  $30^\circ$ ,  $40^\circ$  and  $50^\circ C$  for a period of 30 minutes and the reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. The tubes were cooled immediately in ice-cold water and centrifuged for 10 minutes at 3000 r.p.m. Inorganic phosphate liberated was estimated spectrophotometrically (Beckman DU-2) by the method of Fiske and Subba Row as described by Leloir and Cardini<sup>16</sup>. Protein content in the enzyme extract was estimated by Biuret method<sup>17</sup>.

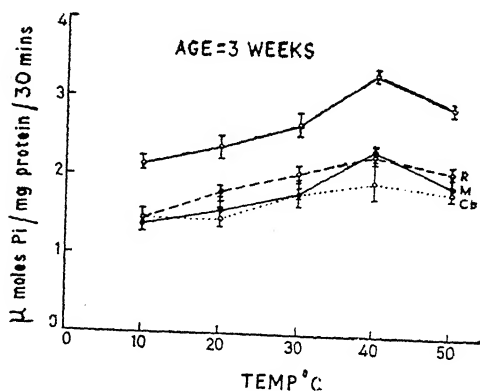
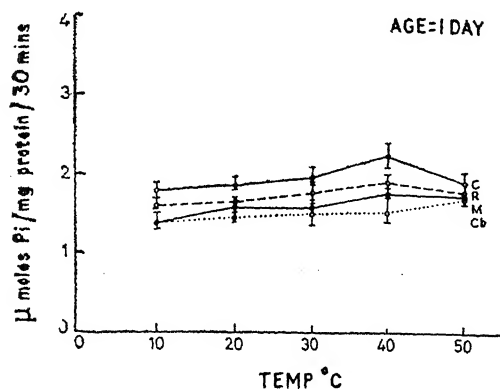
Energy of activation was calculated from the Arrhenius plots as described by Giesels<sup>18</sup>.

### RESULTS

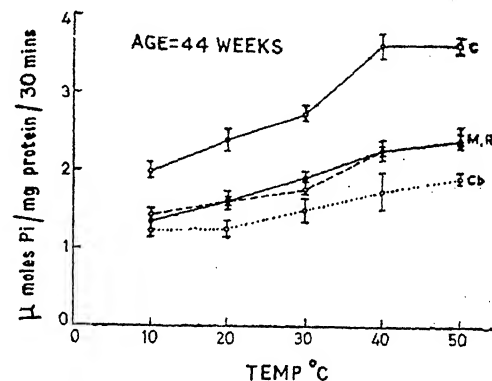
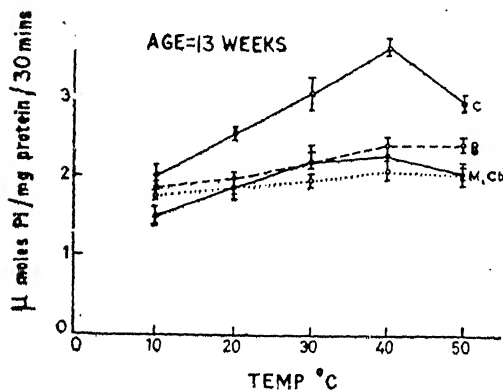
An incubation period of 30 minutes was used for all extracts, regardless of their activity. This period was chosen on the basis of our earlier studies<sup>3</sup>. Figures 1-5 show the activity-temperature relationships of  $Mg^{++}$  ATPase in 1 day, 3, 13, 44 and 87 weeks old rats respectively. It is seen that  $Mg^{++}$  ATPase is temperature insensitive in cerebellum, medulla and optic lobes of 1 day old animals

the temperature range of 10–50°C, whereas the  $\mu$ mc from the cerebrum shows a slight increase the temperature sensitivity in the temperature

range of 30–40°C. The insensitivity in the enzyme activity is noticed in the temperature range of 30–50°C in all the regions of the brain studied



FIGS. 1–2. Fig. 1. Activity-temperature curves for  $Mg^{++}$  ATPase in the CNS of 1 day old rat. fig. 2. Activity-temperature curves for  $Mg^{++}$  ATPase in the CNS of 3 weeks old rat.



FIGS. 3–4. Fig. 3. Activity-temperature curves for  $Mg^{++}$  ATPase in the CNS of 13 weeks old rat. fig. 4. Activity-temperature curves for  $Mg^{++}$  ATPase in the CNS of 44 weeks old rat.

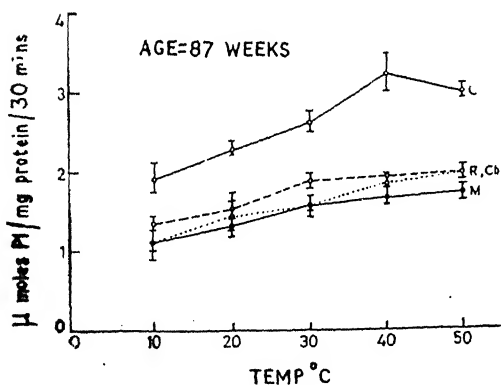


FIG. 5. Activity-temperature curves for  $Mg^{++}$  ATPase in the CNS of 44 weeks old animal. C=Cerebrum; Cb=Cerebellum; M=Medulla; O=Optic lobes.

except in the cerebrum of 87 weeks old rats.  $Mg^{++}$  ATPase of the cerebrum remains temperature sensitive at all the ages studied, the sensitivity being maximum at the age of 13 weeks.

Table I gives the energies of activation of  $Mg^{++}$  ATPase in different regions of the brain. Medulla and cerebrum show only one peak at the age of 3 weeks, whereas in cerebellum and optic lobes two peaks appear in the energy of activation, one at the age of 3 weeks and the other at 44 weeks age, the former peak being higher in both these regions. However, lowest energy of activation in all the regions studied was found in 1 day old animals.

#### DISCUSSION

It is clear from the results that in 1 day old animals the enzyme shows temperature insensitivity



TABLE I  
Energies of activation in the CNS of rats

Age of the animal	Energy of activation Cals/mol			
	Cerebrum	Cerebellum	Medulla oblongata	Optic lobes
1 day	676.3	514.012	1142.515	914.012
3 weeks	3884.5	3327.5	3884.5	2742.0
13 weeks	2056.5	1599.5	2513.5	1371.0
44 weeks	2513.5	2513.5	2513.5	2285.0
87 weeks	1828.0	1828.0	2056.5	1828.0

in the temperature range of 10–50° C in all the regions studied except in the cerebrum where the sensitivity increases in the temperature range of 30–40° C. The temperature sensitivity of the enzyme becomes well defined in 3 weeks old animals in the temperature range of 10–40° C with an activity maxima at 40° C. The temperature insensitivity shown by 1 day old animals in the temperature range of 10–50° C is obviously of importance in the functioning of the central nervous systems of young rats which develop effective thermoregulation only at the age of 2–3 weeks.

In 1 day, 3 weeks and 13 weeks old animals the enzyme shows a steady increase in specific activity with a rise in temperature with a maxima at 40° C in all the four regions studied. Above 40° C, activity either decreases or remains unchanged, whereas in 44 and 87 weeks old animals the peak is shifted in the temperature range of 40–50° C in all the regions. Here the existence of different maxima in the apparent activity of enzyme at different stages of life may be explained by conformational changes in the enzyme, or probably it may be an enzymatically based adaptation of the central nervous system during the aging process.

Studies on the temperature dependence of ATPase (Magnesium stimulated) in the whole brain homogenates of the developing rats by Tirri *et al.*<sup>1</sup> have shown that the maximum activity occurs at 37–41° C and also a discontinuity between 15–20° C. They have also shown a decrease in the temperature sensitivity between 20–30° C in the animals of 1 to 11 days age. The maximum enzyme activity at 40° C during development and the temperature insensitivity in 1 day old animals, shown by Tirri *et al.*<sup>1</sup>, is in conformity with the present results. However, no discontinuity in the enzyme activity as shown by Tirri *et al.*<sup>1</sup> was observed in the present study at any stage of life.

The temperature independence of the enzyme activity in the range of 30–50° C in all the regions of the brain except the cerebrum of 87 weeks old animals is of importance to the aging animals as they tend to lose the capacity of effective thermoregulation.

Studies on the  $Na^{+}$ - $K^{+}$  ATPases which are of importance in the oxidative phosphorylation and active transport, which would throw light on the temperature dependence of the energy transferring processes in the central nervous system of the developing and aging animals, are in progress.

#### ACKNOWLEDGEMENTS

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## GAMETOPHYTES AND SEED DEVELOPMENT IN PINEAPPLE

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THE pineapple, *Ananas comosus* (L.) Merr. of Bromeliaceae consists of herbaceous plants confined mainly to the tropics and subtropics of the new world. The taxonomic details of the genus and the cultivars of pineapple have been adequately reported<sup>1,2</sup>. The different cultivars used in Malaysia are also described<sup>3</sup>. The cultivated pineapple plant is self-incompatible, although it is generally cross-fertile and sporadic cross-pollination occurs in the field with the help of honey-bee and pineapple beetles<sup>4,5</sup>. The details of embryology and seed development in this genus is so far unknown though pineapple is widely grown commercially around the world for more than 50 years<sup>6</sup>. Some of the important points concerning the gametophyte and seed development in *A. comosus* are presented here. Seed structure and germination are described for *A. sativus*<sup>7</sup>.

Artificial crosses were made by hand pollinating the flowers of Masmerah plants with pollen of Mauritius. The post-pollination changes recorded here were determined from the time the flowers were hand pollinated. The pollinated flowers were tagged with date, time and subsequently periodic collections were made. These were fixed immediately and processed for microtoming. In 70% of the crosses made the seeds developed with embryo and endosperm tissue. Customary methods were followed to obtain the paraffin sections and for staining them.

The flowers are zygomorphic and trimerous. The six stamens are arranged in two whorls, the outer epipetalous and the inner epipetalous. The anthers are introse, dorsifixed with elongated pollen sacs. The wall of the young anther is made up of epidermis, endothecium, two middle layers and tapetum (Fig. 1). The tapetal cells are glandular, uninucleate becoming binucleate later on. At maturity the endothecium and a middle layer show fibrous thickenings (Fig. 2). The microspore mother cells undergo the usual reduction division showing dyad and tetrad stages. Separation of microspores takes place by cell plate formation and tetrads show isobilateral arrangement. The mature pollen grains are two celled at the shedding stage.

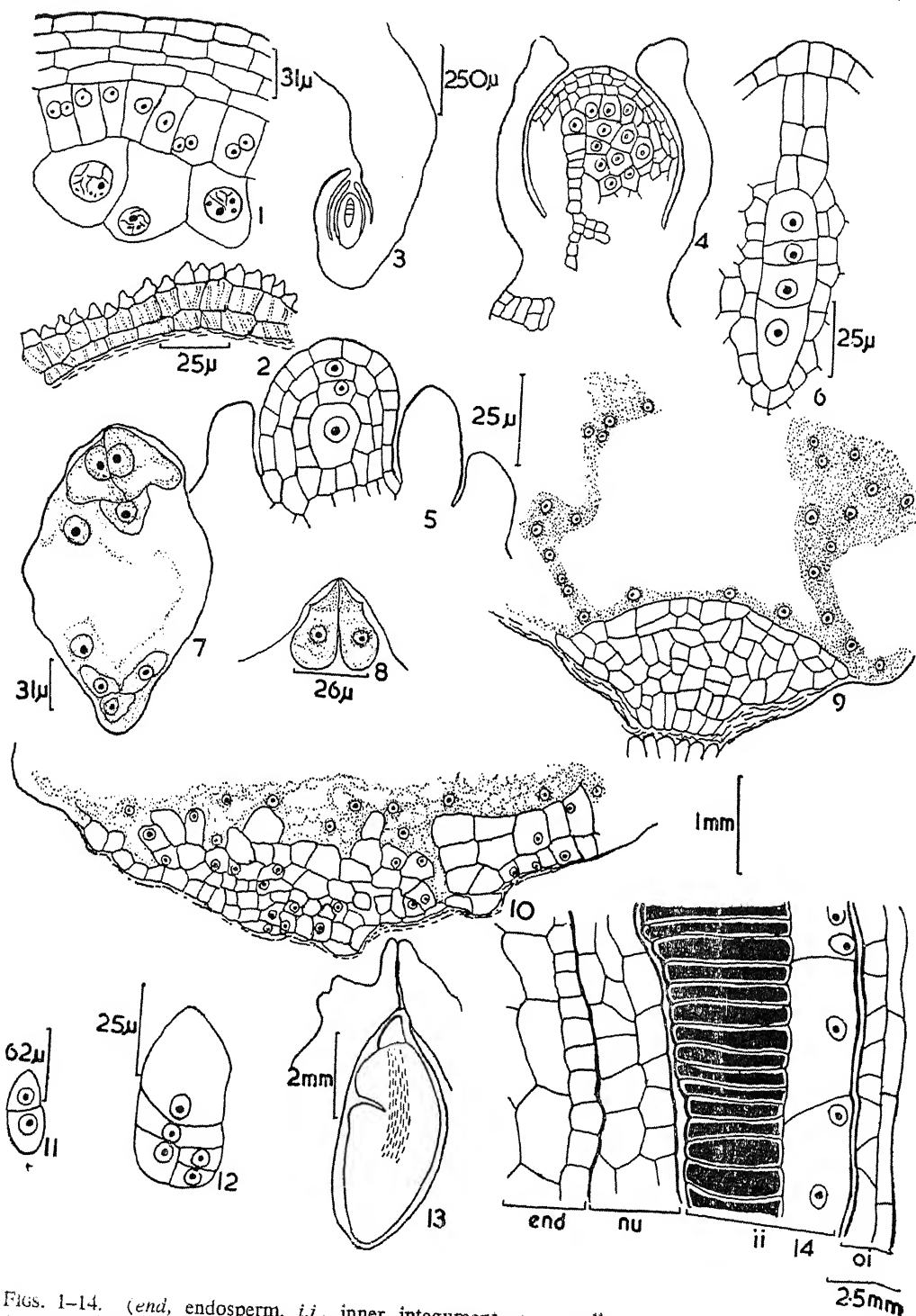
The ovary is inferior, syncarpous, tricarpellary, trilobular and bears 15–20 ovules per locule arranged in axile placentation. The ovules are crassinucellate and mainly anatropous, however, about 3% are of the orthotropous type

(Figs. 3, 4). The anatropous ovules are bitegmic and functional while the orthotropous ovules are unitegmic. In the orthotropous ovules a few of the nucellar cells enlarge to no consequence and the ovules ultimately abort. The subsequent description is based on the development seen in anatropous ovules only.

The archesporial cell is hypodermal and divides periclinally to form primary parietal cell and megaspore mother cell (Fig. 5). The former divides further, either periclinally or anticlinally, before the megaspore mother cell enlarges. The subsequent division of megaspore mother cell results in the formation of dyad and later into a linear tetrad (Fig. 6). Only the chalazal megaspore functions further. The functional megaspore enlarges and the nucleus divides thrice to form the eight nucleate embryo sac of the polygonum type (Fig. 7)<sup>8</sup>. The mature synergids are hooked, pyriform, and show filiform apparatus (Fig. 8). The antipodals are organized as cells and lie in the narrow chalazal end of the embryo sac. They degenerate early, about 12–24 hours after anthesis.

The two polar nuclei meet near the chalazal end or towards the centre of embryo sac. The entry of the pollen tube is porogamous and fertilization takes place within two hours after pollination. About six hours after fertilization the primary endosperm nucleus divides in the chalazal end resulting in a larger micropylar and a smaller chalazal chamber. The nucleus in the micropylar chamber divides repeatedly to form numerous endosperm nuclei which are arranged peripherally. Nucleus in the chalazal chamber divides and soon after cytokinesis sets in organizing a triangular tissue (Fig. 9). Ultimately, the endosperm cells of both micropylar and chalazal regions merge together (Fig. 10). Endosperm formation is thus of the helobial type<sup>8</sup>. The cells of the micropylar region are much bigger than those organized in the chalazal end (Fig. 10). The mature endosperm cells are rich with contents.

The zygote divides transversely resulting in apical (Ca) and basal cells (Cb) (Fig. 11). Ca divides longitudinally and Cb transversely resulting in four celled stage of the embryo. The two cells of the tier Ca divide longitudinally at right angles to the first division giving rise to the quadrant cells (Fig. 12). The derivatives of the quadrant cells and the lower derivatives of Cb give rise to embryo



Figs. 1-14. (end, endosperm, *i.i.*, inner integument, *nu*, nucellus, *o.i.*, outer integument). Fig. 1. young anther wall. Fig. 2. T.S. mature anther wall. Note the persistent middle layer with thickened middle layer and papillose epidermis. Figs. 3, 4. L.S. anatropous and orthotropous ovules respectively. Fig. 5. Ovule showing MMC and parietal cells (4, 5, common scale). Fig. 6. Linear tetrad. Fig. 7. embryo sac. Fig. 8. Synergids showing hooked condition and filiform apparatus. Fig. 9. stages in helobial endosperm development. Figs. 11-12. Young embryo. Fig. 13. Young embryo. Fig. 14. L.S. seed coat.

proper. The upper derivatives of Cb organize the suspensor. The mature embryo is elongated with a distinct cotyledon, and a lateral stem tip (Fig. 13). The embryo development is of the Asterad type<sup>8</sup>.

The mature seed is elongated, broad at one end and pointed at the other. The embryo is situated at the pointed end surrounded by endosperm. The seed coat is dark brown with numerous longitudinal ridges and furrows. The varying thickness of the middle layers results in the formation of ridges and furrows of the seed. Both the integuments contribute towards the formation of seed coat (Fig. 14). Seed germination and seedling development have been studied.

The literature on the embryology of Bromeliaceae has been recently reviewed and only *Tillandsia*, of the 65 genera, has been so far investigated<sup>6</sup>. The major observations made currently compare well with the embryological data presented for the other members of this family. Apart from the data recorded above, other variations are noticed in pollen germination, in the development of the ovule, embryo sac, the behaviour of polar nuclei as well as endosperm formation. Such variations are mostly confined to sterile hybrids as well as their abortive seeds and these will be discussed in detail in a subsequent paper. Occurrence of two types

of ovules in the same species has been reported in members of Ranunculaceae and Valerianaceae<sup>9,10</sup>.

It is to be noted that cytokinesis sets in the chalazal chamber earlier than in the micropylar chamber (Fig. 9). Thus the sequence of endosperm formation observed in this species is different from the developmental changes recorded for other monocots<sup>11</sup>.

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## LETTERS TO THE EDITOR

HOLOGRAPHIC INTERFEROMETRY;  
MEASUREMENT OF IN-PLANE DEFORMATION

HOLOGRAPHIC Interferometry, now-a-days, has become a powerful tool to study distortion of structure under load, effects of creep, fatigue, buckling or thermal distortion. This method added extra capability to the existing conventional interferometry by relaxing some of the exacting requirements<sup>1-4</sup>.

To investigate the in-plane deformation an annular ring is selected in view of its special nature involving discontinuities within. With a specially made apparatus, the ring is subjected to known diametrically compressive force. Double-exposure hologram is recorded for three different loads with the usual off-axis arrangement. With equal duration of exposures before and after the specimen is loaded the 'frozen' fringes are produced on the reconstruction of the hologram. These fringes are photographed for the purpose of measurement. One such pattern for the load of 1.5 Kg is shown in Fig. 1.

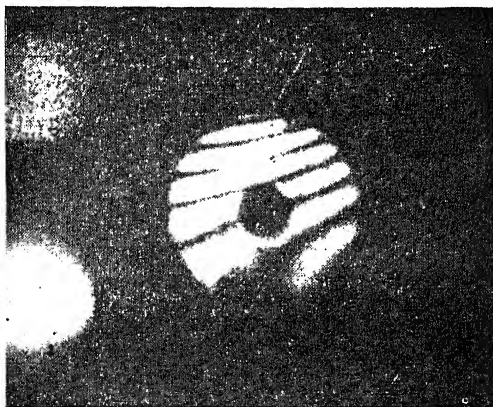


FIG. 1. 'Frozen' fringe pattern with 1.5 kg load.

Fringe order is assigned assuming the fringe formed at the bottom resting point of the ring as the zeroth one for at this point the deformation is considered zero. If P is a representative point before loading, on the vertical diameter of the specimen and P' is the position it assumes a distance  $d$  away on compression, the path difference,  $\Delta$ , produced is

$$\Delta = d(\cos \theta_i - \cos \theta_s) \quad (1)$$

where  $\theta_i$  is the illuminating angle and  $\theta_s$  is the scattering angle both made with the plane of the

member. The order of the fringe N can be given by

$$d(\cos \theta_i - \cos \theta_s) = N\lambda$$

where  $\lambda$  is the wavelength used. If  $d'$  is the maximum linear displacement occurred at the point of loading and  $N_{\max}$  is the order number at the point, we have

$$d' = \frac{N_{\max} \lambda}{(\cos \theta_i - \cos \theta_s)} \quad (2)$$

Employing this formula the in-plane deformations at this point are calculated and the results are given in Table I.

TABLE I

Load applied (Kg)	Order of the fringe at the topmost point	Deformation $d'$ (Cm)
1.5	6	$4.15 \times 10^{-4}$
3	12	$8.29 \times 10^{-4}$
3.5	18	$12.45 \times 10^{-4}$

The possible errors that may creep into the results are due to (1) diverging nature of the illuminating laser beam, (2) difficulty in estimating the fraction of the fringe and (3) inaccuracy in the measurement of the angles  $\theta_i$  and  $\theta_s$ . After minimizing the effects produced on these accounts the total error estimated is about 12%.

The correlation of these results with those of theoretical values which are being calculated, and the experimental details will be published elsewhere.

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# EFFECT OF MAGNETIC ACTIVITY ON THE FADING OF RADIO WAVES AT WALTAIR

RAO AND RAO reported from a preliminary study, that the E-region fading frequency was higher during magnetically disturbed days, when compared to that of quiet days. In this paper the effect of magnetic activity on radiowave fading is studied in more detail using the data taken during IGY at Waltair (Geomag. Lat.  $7.4^{\circ}$  N).

K-indicies of Alibag magnetic observatory (Geomag. Lat.  $9.5^{\circ}$  N) were allotted for the entire fading data. Average values of the fading frequency were then obtained for each particular value of the K-index for the entire period. In order to study the effect of magnetic activity on the day time (0700–1800 Hrs) and night time (1900–0600 Hrs) fading frequencies separately, average day time and night time fading frequencies for different values of K-index, were also calculated. In Fig. 1

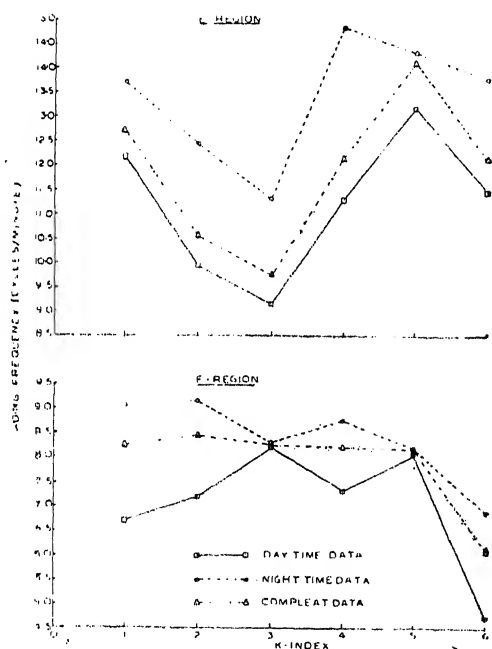


FIG. 1. Variation of average fading frequency with K-index.

the variation of the average value of fading frequency with K-index for day time, night time and complete data, for both E- and F-regions, were plotted.

It can be seen from the figure that the general trend of variation of average fading frequency with K-index is almost the same for day time and night time in the case of E- and F-regions. Statistical methods were used and the correlation coefficient between K-index and fading frequency was found

to be insignificant in both E- and F-regions. To study this aspect in more detail, the average values of the fading frequency corresponding to the lower K-indicies range ( $K = 1$  to  $3$ ) and higher K-indicies range ( $K = 4$  to  $6$ ) were obtained separately and presented in Table I.

TABLE I

Average fading frequency in E- and F-region during different periods

Sl. No.	Region	Average fading frequency (cycles/minutes in)			
		Lower K-indicies range		Higher K-indicies range	
		Day time	Night time	Day time	Night time
1.	E-Region	10.49	12.71	11.53	14.54
2.	F-Region	7.40	8.90	7.03	8.32

From a perusal of Table I, it is found that the fading frequency increased during higher K-index range, when compared to the lower K-index range in the E-region during both day and night times, indicating the effect of magnetic activity is to increase the fading frequency. It can also be seen from the table, that the increase of fading frequency, during higher K-indicies range is more during night time, than during day time, in the E-region. On the contrary in the F-region, the average value of the fading frequency is slightly less during higher K-indicies range, when compared to the lower K-indicies range indicating that there is no significant effect of magnetic activity on the F-region fading.

From Fig. 1 it can also be seen that the night time fading frequencies are greater than day time fading frequencies in both E- and F-region, during the entire K-indicies range, the increase being greater in the case of E-region. This increase of fading frequency in the E-region during night time might be partly due to the variation produced by meteors (Rao and Rao<sup>2</sup>).

In the case of F-region, this increase of fading frequency during night time may be attributed to (1) the presence of spread F irregularities and (2) larger drift speeds during night time than day time. Rao and Rao<sup>1</sup> reported a negative correlation between the F-region frequency and magnetic activity, while Patel found no significant correlation between the magnetic activity and the day time F-region fading frequency. The results of the present study are in agreement with the findings

of Patel<sup>4</sup>. The observed increase of fading frequency during night time in the E-region, is in conformity with the results of Millman<sup>5</sup>.

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TABLE I

Relative intensities of gamma-rays in the  
decay of  $^{125}\text{Sb}$

Gamma Energy (keV)	Relative intensities		
	Present results	Marsol and Ardisson <sup>2</sup>	J. B. Gupta <i>et al.</i> <sup>3</sup>
35.6	$1.42 \pm 0.086$	..	..
81.8	..	..	$< 0.02$
100.1	$0.36 \pm 0.04$	0.045	..
111	$0.17 \pm 0.023$	$< 0.02$	$< 0.01$
116.8	$0.96 \pm 0.07$	$1.13 \pm 0.11$	$0.35 \pm 0.05$
172.7	$0.47 \pm 0.03$	$0.89 \pm 0.1$	$0.72 \pm 0.05$
176.6	$23.2 \pm 1.32$	$22.7 \pm 0.9$	$22.8 \pm 0.4$
178.8	$0.05 \pm 0.01$	..	$0.08 \pm 0.01$
193.6	..	..	$0.04 \pm 0.01$
204.2	$1.1 \pm 0.03$	$0.93 \pm 0.09$	$1.14 \pm 0.03$
208	$0.83 \pm 0.05$	$0.63 \pm 0.06$	$0.77 \pm 0.05$
227.6	$0.64 \pm 0.04$	$0.39 \pm 0.04$	$0.44 \pm 0.02$
321.1	$1.6 \pm 0.1$	$1.52 \pm 0.15$	$1.43 \pm 0.04$
380.1	$5.43 \pm 0.32$	$5.1 \pm 0.3$	$5.12 \pm 0.1$
408	$0.5 \pm 0.032$	$0.45 \pm 0.05$	$0.55 \pm 0.03$
427.6	100	100	100
443.3	$1.1 \pm 0.07$	$1 \pm 0.2$	$1.05 \pm 0.03$
463.2	$35.2 \pm 2.3$	$35 \pm 1.5$	$35.1 \pm 0.6$
489.8	..	..	$< 0.01$
600.5	$53.6 \pm 3.2$	$59.8 \pm 2.5$	$60.2 \pm 0.9$
606.6	$19 \pm 1.1$	$16.4 \pm 0.8$	$16.7 \pm 0.3$
636.1	$35.6 \pm 2.3$	$38.4 \pm 1.9$	$38.5 \pm 0.6$
671	$6.24 \pm 0.38$	$5.83 \pm 0.3$	$6.05 \pm 0.12$

#### ON THE DECAY OF $^{125}\text{Sb}$

ALTHOUGH the level structure of  $^{125}\text{Te}$  was extensively investigated through the beta decay of  $^{125}\text{Sb}$  as well as nuclear reaction studies, there are still some uncertainties on the existence or otherwise of a few gamma lines as reported in the recent summary of Auble<sup>1</sup> in Nuclear Data Sheets. The present study is therefore motivated for a careful search of the seven disputed gamma-rays shown by dotted lines in the drawing of the above reference. The radioactive source  $^{125}\text{Sb}$  is obtained as antimony chloride and oxychloride in dilute HCl with a concentration of about 6 mCi in 5 ml. Three such samples obtained at different times are employed to check for the reproducibility of the results and any possible impurities. A Ge(Li) detector system, consisting of a 35 c.c. Ge(Li) coaxial detector, SF 100 C pre-amplifier, PA 563 amplifier and Nuclear Data 512 channel analyser, is employed in the work. A bias-amplifier type BA 669 is used to work for different parts of the spectrum in detail. The system resolution is found to be 4 keV at 662 keV. The system is initially calibrated for linearity and relative photopeak efficiency, using sources  $^{152}\text{Eu}$  and  $^{133}\text{Ba}$ . The spectrum is recorded in several overlapping regions with adequate scale expansion and counting times to search for the suggested weak lines. The peaks were gaussian fitted on an IBM 1130 computer and the energies and relative intensities recorded in the different spectra are obtained.

The results are furnished in Table I together with those of the recent investigations.

It can be seen from Table I that in the present work 20 gamma-rays are indicated in agreement with Gupta *et al.*<sup>3</sup>. However, while they found

evidence for a 199 keV line and ruled out 111 keV line, in the present study evidence is obtained for the 111 keV line ruling out the 199 keV line. In both studies, evidence is obtained for the 179 keV line. Both studies ruled out disputed gamma-rays at energies 82, 122 and 489 keV. There is an overall agreement among the results for the relative intensities.

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### ACID-CATALYSED REACTION OF ACETOPHENONE OXIME WITH 2,4-DINITROPHENYLHYDRAZINE

We describe in this communication the direct instantaneous reaction of acetophenone oxime with 2,4-dinitrophenylhydrazine in acid medium. The reaction is acid-catalysed and in the absence of  $H^+$ , the reaction does not proceed at all. It is not the hydrolysis of the oxime, catalysed by  $H^+$ , which precedes the reaction. But  $H^+$  functions as the catalyst in the actual reaction. The sparingly soluble and non-volatile nature of 2,4-dinitrophenylhydrazones makes this reaction suitable for the quantitative estimation of oximes.

Except the preliminary work of Brady and Peakin<sup>1</sup> in 1929, no work has been done so far, on the reaction between oxime and 2,4-dinitrophenylhydrazine. We have investigated this reaction taking acetophenone oxime as the typical oxime.

The following experiment illustrates the simplicity of the method employed. Acetophenone oxime (0.02 to 0.4 gm) dissolved in acetic acid is treated with excess of Borsche's reagent (2,4-dinitrophenylhydrazine in 2N HCl). The reaction occurs virtually instantaneously at room temperature to give 2,4-dinitrophenylhydrazone. After allowing the reaction mixture to stand for a few minutes, the Borsche's derivative is filtered, washed with 2N HCl and then with water. The precipitate is dried at 100°C, cooled and weighed. The m.p. (237°C, literature value<sup>2</sup> = 237°C) and *tlc* taken correspond to those of acetophenone 2,4-dinitrophenylhydrazone, prepared separately from the carbonyl compound. The weight of the derivative obtained from the oxime showed that the oxime is quantitatively converted to the Borsche's derivative (Table I).

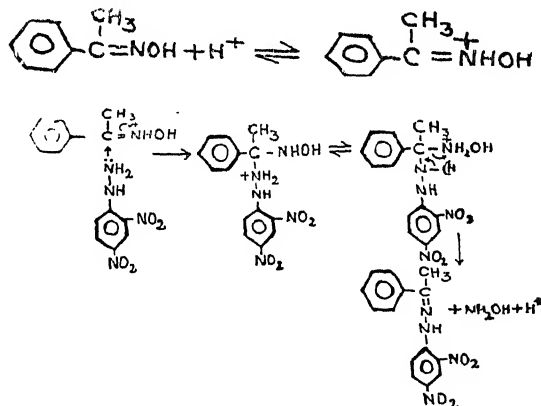
From the experimental results, we conclude the following:

(1) There is no reaction between acetophenone oxime and 2,4-dinitrophenylhydrazine in the absence of  $H^+$ . This has been arrived at by

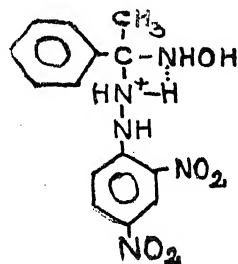
TABLE I

Weight of acetophenone oxime (originally taken) in gm	Estimated value in gm (from Borsche's derivative)	Percentage yield
0.0295	0.0294	99.65
0.0590	0.0586	99.31
0.1180	0.1170	99.15
0.2935	0.2930	99.84

conducting the reaction in acetonitrile and taking *tlc* for the reaction mixture. (2) The reaction is acid catalysed.  $H^+$  does not bring about the hydrolysis of the oxime prior to the reaction of the latter with the nucleophile. It catalyses the reaction between the oxime and 2,4-dinitrophenylhydrazine, a weak nucleophile. Similar acid-catalysis has been observed in carbonyl additions<sup>3,5</sup> involving weak nucleophiles. Based on the reaction pathways for carbonyl additions<sup>3,5,6</sup> and reactions of Schiff's bases<sup>4</sup>, the mechanism of the reaction can be depicted as follows:



In the oxime, nitrogen being more electronegative than carbon polarizes the double bond and this leaves a partial positive charge on carbon to facilitate nucleophilic attack. 2,4-dinitrophenylhydrazine being a weak nucleophile requires an enhancement of positive charge on carbon which is brought about by the protonation of nitrogen. The transition complex may be stabilized by intramolecular hydrogen-bonding as





(3) The reaction is practically quantitative. The yield of 2, 4-dinitrophenylhydrazone is  $> 99\%$  (Table I) and hence the reaction can be employed as a method for the estimation of small amounts of oximes which find use in many fields as catalysts, photoinitiators, swelling, antimalarial and antiskinning agents, rocket fuels, etc.

(4) In studies involving the acid-catalysed or oxidative hydrolysis of oximes, the carbonyl compound cannot be estimated as its Borsche's derivative before separation of the carbonyl compound from the reaction mixture as both the oxime and carbonyl compound would react with Borsche's reagent.

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## SALICYLIC ACID AND ITS RELATED COMPOUNDS AS INDICATORS FOR THE DIRECT EDTA TITRATION OF FERRIC IONS

### ABSTRACT

Salicylic acid, salicylaldehyde, methyl salicylate, ethyl salicylate and phenyl salicylate have been used as indicators for the direct EDTA titration of ferric ions. Titrations can be accurately carried out in the pH range 3.0–3.0. A large number of diverse ions are tolerated. Several synthetic solutions have been analysed according to the recommended procedure.

ORTHOHYDROXY ketones and their oximes<sup>1</sup>, proto-catechualdehyde and its related compounds<sup>2</sup>, cinnamohydroxamic acid, *n*-sulfophenyl hydroxamic acid, *n*-benzoyl-*n*-phenyl hydroxylamine and *n*-phenyl hydroxamic acid<sup>3</sup>, 4-OH-1-*p*-sulfonate phenyl triazine<sup>4</sup> and a number of substances have been used as indicators for direct EDTA titration of ferric ions, while salicylic acid and its related compounds have not been investigated as indicators. This article reports the use of salicylic acid and its related compounds as indicators for the direct titration of ferric ions with EDTA solution in burette.

### EXPERIMENTAL

**Ferric Nitrate Solution.**—A stock solution of ferric nitrate (0.1 M) was prepared by dissolving 40.38 gm ferric nitrate non-hydrate in distilled water and making up to 1 liter. It was standardised gravimetrically as ferric oxide.

**EDTA Solution.**—A stock solution of 0.01 M Na-salt of EDTA was prepared by dissolving 3.723 mg of EDTA in 1 liter of distilled water.

**Indicator Solutions.**—1% solutions were prepared from pure samples of the reagents by dissolving the requisite amount in 95% alcohol. The solutions were stable up to four weeks.

**Metal Solutions.**—To study the interference of various foreign ions in the estimation of  $\text{Fe}^{3+}$  with EDTA, the corresponding analar quality of salts were taken and their 0.2 M solutions prepared in distilled water.

### OBSERVATIONS

Salicylic acid and its related compounds give a violet colour with ferric ions. There is a sharp colour change on titration with EDTA from violet to yellow as the end point approaches.

**Effect of pH.**—When an aliquot of  $\text{Fe}^{3+}$  is titrated with EDTA, iron can be estimated accurately in the pH range 2.0–3.0 with the help of the indicators investigated.

**Effect of Indicator Concentration.**—Titrations were carried out with different concentrations of the indicators. It was found that an addition of 5 drops of 1% solution of salicylic acid and salicylaldehyde gave satisfactory results. While 1–2 ml of 1% solution of methyl salicylate, ethyl salicylate and phenyl salicylate gave slightly variable results.

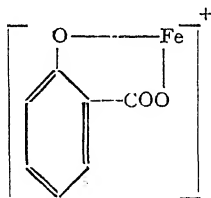
**Effect of Temperature.**—The titration could be satisfactorily carried out in the temperature range 10°–60° C.

**Effect of Foreign Ions.**—To investigate the effect of foreign ions, titrations of synthetic solutions were carried out at pH 2.0, maintaining the pH by acetate—HCl buffer. At 2.8 mg concentration of iron, 50 times excess of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ , 10 times excess of  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ , 5 times excess of  $\text{Ag}^+$ ,  $\text{Al}^{3+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$  and 2 times excess of  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  could be tolerated. Anions forming stable complexes with  $\text{Fe}^{3+}$  interfered at all levels. Similar observations with other indicators have been reported by Desai and co-workers<sup>5,6</sup>.

**Suggested Procedure.**—Take a 5.0 ml aliquot of solution containing ferric ions, adjust pH to 2.0–3.0 using acetate—HCl buffer of pH 2.0, add 5 drops of 1.0% solution of indicator and titrate against 0.01 M solution of EDTA to light yellow colour.

**Standard Deviation.**—Five, 5.0 ml aliquots of 0.01 M ferric nitrate were analysed according to the suggested procedure. The standard deviation was 0.5% for salicylic acid and salicylaldehyde, while it was 2% for methyl salicylate, ethyl salicylate and phenyl salicylate.

Salicylic acid reacts with ferric ions to form a chelate having the following structure :



Addition of EDTA, destroys the complex.

Thanks are due to the Principal, Borsad Science and Law College and E.M.H.S., Trust, Borsad, for extending laboratory facilities.

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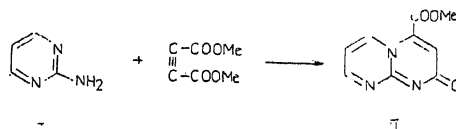
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#### A NOVEL SYNTHESIS OF METHYL 4H-PYRIMIDO [1, 2-*a*] PYRIMIDIN-2-ONE- 4-CARBOXYLATE

ONLY few reports are available for the synthesis of pyrimido 2,2-*a* pyrimidines<sup>1-4</sup>. The present communication reports a simple and novel synthesis of methyl 4H-pyrimido [1, 2-*a*] pyrimidin-2-one-4-carboxylate.

Dimethyl acetylene dicarboxylate in methanol was added to a stirred solution of 2-aminopyrimidine (I) in ice salt bath. The mixture was stirred over-night at room temperature. A light yellow crystalline solid m.p. 178° (yield 70%) was obtained. The structure (II) of the product was established on the basis of spectral and analytical data. The reaction is presumed to proceed through a Michael type adduct intermediate which loses a molecule of methanol to finally yield (II).

Anal. (%) calculated for C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>3</sub> : C, 52.68 ; H, 3.41 ; N, 20.48. Found : C, 52.60 ; H, 3.12 ; N, 20.53. Molecular weight = 205. U.V. (Ethanol, nm), 345, 248. I.R. (Nujol, cm<sup>-1</sup>): 3060 m, 1740 s (ester), 1705 s (C=O), 1625 s (C=N), 1540 w, 1525 w, 1490 w, 1320 s (C-N), 1155 m, 1145 w, 1110 m, 995 s, 795 m. N.M.R. (60 MHz, CDCl<sub>3</sub>, TMS, δ values): 7.40 (m, 2H), 4.10 (s, 3H, OCH<sub>3</sub>) and 9.25 (broad m, 2H).



From the above data the product was assigned the structure (II) methyl 4H-pyrimido [1, 2-*a*] pyrimidin-2-one-4-carboxylate.

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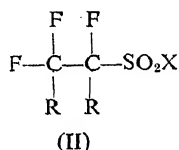
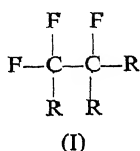
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#### INTERACTION OF BISTRIFLUOROMETHYL NITROXIDE WITH PERFLUOROVINYL- SULPHONYL HALIDES

DURING attempts to prepare a self-curing elastomer related to 'Viton A'<sup>1-3</sup>, perfluorovinylsulphonyl chloride<sup>4</sup> was evaluated as a monomer in copolymerisations with vinylidene fluoride, under different experimental conditions<sup>5</sup>. These polymerisations invariably led to liberation of small amounts of SO<sub>2</sub> and formation of low-molecular weight liquid polymers, while a white solid copolymer could be obtained from the related monomer, perfluorovinylsulphonyl fluoride<sup>6</sup> and vinylidene fluoride. The failure to get a high-molecular weight copolymer from perfluorovinylsulphonyl chloride was attributed to competition from a facile chain transfer process involving abstraction of Cl by a growing polymer radical or the initiator radical followed by the

liberation of  $\text{SO}_2$ . Similar abstraction of Cl by isobutyronitrile radical had been observed earlier<sup>4</sup>.

Thus, it was of interest to study the interaction of the known stable free radical, bistrifluoromethyl nitroxide<sup>7,8</sup> with perfluorovinylsulphonyl chloride and also with perfluorovinylsulphonyl fluoride to ascertain if the radical would abstract Cl from perfluorovinylsulphonyl chloride leading eventually to substitution of the  $-\text{SO}_2\text{Cl}$  group. It was however realised that a species like  $(\text{CF}_3)_2\text{NOCl}$  might not be stable enough to be isolated; but initial liberation of  $\text{Cl}_2$  and  $\text{SO}_2$  might still occur leading to substitution along with the normal addition of the radical to the double bond and resulting in the formation of I or/and II a.



[R =  $(\text{CF}_3)_2\text{NO}-$ ; (II a), X = Cl; (II b), X = F]

The reactions were carried out by condensing the olefinic compound and excess bistrifluoromethyl nitroxide *in vacuo* into thick-walled pyrex reaction tubes cooled in liquid nitrogen. The tubes were sealed and the contents allowed to react at the desired temperature. The products were separated by low temperature fractionation in a conventional all-glass vacuum system and characterised by spectral data and elemental analysis. From the reaction of perfluorovinylsulphonyl fluoride (3.05 mmoles) with bistrifluoromethyl nitroxide (12.2 mmoles) at  $20^\circ\text{C}$  for 48 hours, a colourless liquid was isolated in 91% yield. It was found to be a pure compound by g.l.c. (2 m SE-30 at R.T. and at  $50^\circ\text{C}$ , and 2 m Apiezon-L at R.T.) and was identified as the expected perfluoro [1,2-bis(N,N-dimethylamino-oxy)ethane] sulphonyl fluoride (II b). (Observed: C, 14.5; H, 0.1; N, 5.7%.  $\text{C}_6\text{F}_{16}\text{N}_2\text{SO}_4$  requires: C, 14.4; H, 0.0; N, 5.6%). B.P.  $121^\circ\text{C}/752$  mm Hg (Siwoloboff). Its i.r. spectrum showed absorptions at  $6.95\mu$  ( $\text{SO}_2\text{F}$ ), strong absorption bands at  $7.58-8.50\mu$  (C-F str.),  $9.25\mu$  (N-O str.),  $10.35\mu$  (C-N str.) and  $14.0\mu$  ( $\text{CF}_3$  def.). Its  $56.46$  Mc/sec.  $^{19}\text{F}$  n.m.r. spectrum (relative to external  $\text{CF}_3\text{COOH}$ ) had four absorption systems at  $-126$  ppm (broad, complex) attributed to the F in  $-\text{SO}_2\text{F}$ , a triplet ( $J=7.3$  Hz) at  $-9.0$  ppm with an ill-resolved side band at  $-9.5$  ppm [ $(\text{CF}_3)_2\text{NO}-$ ], a broad singlet at  $+7.0$  ppm ( $\text{CF}_2$ ) and a doublet of triplets ( $J=14.9$  Hz and  $5.6$  Hz) centred at  $+38.0$  ppm (CF) in the approximate ratio 1:12:2:1.

Reaction of perfluorovinylsulphonyl chloride (3.82 mmoles) with bistrifluoromethyl nitroxide (15.28 mmoles) at  $20^\circ\text{C}$  for 48 hours, gave only one product as shown by g.l.c. (2 m SE-30 at R.T. and at  $50^\circ\text{C}$ ). This colourless liquid was isolated in 96% yield and was identified as perfluoro [1,2-bis(N,N-dimethylamino-oxy-ethane)] sulphonyl chloride (II a). (Observed: C, 14.1; H, 0.2; N, 5.3; F, 54.8%.  $\text{C}_6\text{F}_{15}\text{N}_2\text{SO}_4\text{Cl}$  requires: C, 13.9; H, 0.0; N, 5.4; F, 55.2%). B.P.  $155^\circ\text{C}/758$  mm Hg (Siwoloboff). Its i.r. spectrum showed strong absorption bands at  $7.15\mu$  ( $\text{SO}_2\text{Cl}$ ),  $7.5-8.5\mu$  (C-F str.),  $9.35\mu$  (N-O str.),  $10.3\mu$  (C-N str.) and  $14.0\mu$  ( $\text{CF}_3$  def.). Its  $56.46$  Mc/sec.  $^{19}\text{F}$  n.m.r. spectrum ( $\text{CF}_3\text{COOH}$  as external reference) was consistent with the structure and had absorptions at  $-11.0$  ppm (triplet,  $J=7.3$  Hz) with an ill-resolved band at  $-11.5$  ppm [ $\text{CF}_3$  in  $(\text{CF}_3)_2\text{NO}-$ ], a broad singlet at  $+3.0$  ppm due to  $\text{CF}_2$  and a broad multiplet at  $+34$  ppm (CF) in the ratio 12:2:1. No  $\text{SO}_2$  or  $\text{Cl}_2$  or (I) could be detected in the reaction mixture indicating that the  $-\text{SO}_2\text{Cl}$  group has been unaffected by the nitroxide radical. A separate reaction carried out with  $\text{CF}_3\text{CFH}.\text{SO}_2\text{F}$ <sup>9</sup> and bistrifluoromethyl nitroxide in the ratio 1:2 at  $90^\circ\text{C}$  for 5 days resulted in the recovery of the starting materials almost quantitatively indicating that even hydrogen abstraction did not take place under these conditions. This in itself is interesting as bistrifluoromethyl nitroxide is known to abstract hydrogen from saturated hydrocarbons<sup>8</sup>.

The above represents part of the work carried out at the Department of Chemistry, University of Manchester Institute of Science and Technology, Manchester, U.K., during the tenure of a Commonwealth Scholarship.

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CHEMICAL CONSTITUENTS OF THE SEEDS  
OF *ENTADA SCANDENS* BENTH.

*Entada scandens* Benth. (Leguminosae), is a large climber of the forests. The seeds are reputed in Indian medicine as a tonic, emetic, anti-periodic and anthelmintic<sup>1</sup>. From the seed kernels Dutta<sup>2</sup> reported the presence of two saponins and Barua *et al.*<sup>3</sup> isolated a new acid saponin, entagenic acid in addition to oleanolic acid, echinocystic acid and methyl mercaptan. The pericarps have not so far been chemically examined.

The powdered pericarps were extracted exhaustively with boiling alcohol. The combined extract was concentrated and the dark brown residue was fractionated into petrol, ether, ethyl acetate and *n*-butanol soluble fractions. The petrol fraction on evaporation and chromatography over silica gel gave four compounds. Two of them were identified as  $\beta$ -sitosterol and  $\alpha$ -amyrin by comparing them and their acetates directly with authentic samples (m.m.p. and TLC). The third compound (TLC;  $R_f$ , 0.36 in benzene-ethyl acetate 20:1) gave a sky-blue fluorescence in UV light. It did not get acetylated showing the absence of hydroxyl function and did not react with ethereal diazomethane. The fourth compound (paper chromatography;  $R_f$ , 0.88 in 60% HOAc) gave blue ferric colour. Further studies are in progress to characterise these two compounds.

The ether extract on evaporation left a residue which gave positive tests for flavonoids. On column chromatography over silica gel, three compounds were isolated. Two of them were identified as quercetin and gallic acid by direct comparison with authentic samples (paper chromatography in four solvent systems). The third compound gave blue ferric colour and was identical with the compound mentioned earlier (paper chromatography;  $R_f$ , 0.88 in 60% HOAc).

The residues from ethyl acetate and *n*-butanol fractions gave typical proanthocyanidin colour reactions. A light brown solid was isolated from the residue of ethyl acetate fraction and it was converted into the flavylium salt by heating with alcoholic hydrochloric acid. It was rose-red in colour and had absorption maximum at 538 nm in methanol which shifted to 550 nm on the addition of aluminium chloride. These indicated that the flavylium salt may be cyanidin chloride which was confirmed by comparing it with authentic cyanidin chloride (paper chromatography; two systems). The *n*-butanol extract did not give any aglycone on boiling with 10% sulphuric acid showing the absence of any glycosides.

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A NOTE ON TOLERANCE TO LEAF RUST  
(*PUCCINIA RECONDITA* ROB. EX DESM.)  
OF WHEAT

MANY wheat varieties, despite sharing heavy infection of rusts, were observed to give high yields in India. It was, therefore, suspected that tolerance mechanism is operating in such varieties. Tolerance in wheat to leaf rust (*Puccinia recondita*) has been reported by many workers abroad (Salmon and Laude, 1932; Caldwell *et al.*, 1934; Peturson *et al.*, 1948; Roberts, 1971). Tolerance to leaf rust of barley has also been demonstrated (Newton *et al.*, 1945; Caldwell *et al.*, 1958; Simons, 1965, 1966).

The purpose of this study was to ascertain tolerance to leaf rust in commercial and future wheat varieties, evolved in India.

Of the seventy-four varieties included in 'Trap Nurseries' under All-India Wheat Improvement Programme (Plant Pathology) in 1972-73, 30 varieties were evaluated for their tolerance to leaf rust. Simple linear regression analysis was used to relate grain weight and yield per plant to disease coefficient. The per cent of loss in grain weight or yield/plant was estimated from regression line as per procedure adopted by Nema and Joshi (1972).

The disease coefficient was treated as independent variable and grain weight or yield/plant as dependent variable. The percentage of rust intensity and the type of reaction was recorded on March 12, 1973 according to modified Cobb's scale. The coefficient of infection was calculated by multiplying the percentage of infection by a response value assigned to each reaction type (Loegering, 1959).

In 18 varieties, the disease coefficient of 1.80 to 40 brought reduction in grain weight by 0.02 to 13.48% and yield losses/plant were 0.30 to 13.06%. However, variety Hira with 40% disease did not show any loss either in grain weight or yield/plant (Table I).

The remaining 12 varieties had disease coefficient of 60-100. Five of these, viz., MACS-9/Kalyan

TABLE I

Mean coefficient of infection per cent loss in grain weight and yield/plant in wheat varieties infected with leaf rust (*Puccinia recondita* Rob. ex Desm.)

S. No.	Variety	Mean coefficient of infection	Loss in 1,000 grain weight %	Per plant loss in yield %
1.	MACS-9	96.50	47.24	59.22
2.	HD-4502	40.00	12.18	14.22
3.	HD-1739	25.00	5.83	8.76
4.	Safed Lerma	1.80	5.40	6.21
5.	Chhoti Lerma	9.00	3.60	4.80
6.	Sharbati Sonara	39.00	4.30	4.02
7.	Hira	40.00	0.00	0.00
8.	Moti	93.00	0.00	0.00
9.	Kiran	9.00	3.55	2.00
10.	Narbada-4	40.00	2.55	1.50
11.	K-68	93.00	2.87	3.64
12.	HD-1925	24.50	7.81	4.54
13.	HD-1982	25.00	12.83	17.15
14.	HD-2009	4.00	13.48	13.06
15.	HD-2012	24.00	1.25	0.30
16.	HD-4513	1.80	9.83	10.30
17.	Pusa Lerma	9.00	0.00	0.00
18.	HD-4530	66.50	0.00	0.00
19.	EK-69	64.50	0.00	0.00
20.	J-1-7	100.00	0.00	0.00
21.	NP 200	100.00	0.00	0.00
22.	Kalyan Sona	96.50	32.00	25.16
23.	Sonalika	67.00	27.23	16.83
24.	Agra local	93.00	43.63	57.58
25.	HP-916	64.50	0.00	0.00
26.	UP-310	4.50	11.03	4.47
27.	UP-319	4.00	5.84	1.12
28.	WL 208	68.00	15.04	16.19
29.	WL 303	25.00	9.34	14.70
30.	Raj 821	9.50	0.02	0.03

Sona, Sonalika, Agra local and WL 208 had the per cent loss in grain weight varying between 15.04 to 47.24 and that in yield/plant from 16.19 to 59.22. The other six varieties, viz., Moti HD-4530, EK-69, J-1-7, NP 200 and HP-916 did not show any loss in grain weight or yield/plant. In K-68 with disease coefficient of 93.00, the loss in grain weight was 2.87% and reduction in yield/plant was 3.64.

It was concluded that there was a direct relationship between yield and leaf rust except in varieties Moti, HD 4530, EK 69, J-1-7, NP 200, HP 916, Hira and K-68 and therefore, these varieties are rated as tolerant.

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#### AN AUTO-TETRAPLOID IN RICE (*ORYZA SATIVA* L.) INDUCED BY EMS

AN auto-tetraploid was obtained in one of the treatments with the potent alkylating mutagen, Ethyl Methane Sulfonate (EMS), in experiments conducted on the effect of alkylating and non-alkylating chemical mutagens on different varieties and hybrids of rice (*Oryza sativa* L.)

The 'mutant' was screened at the  $M_1$  generation in one of the experiments, from an *indica* × *indica* (TN-1 × MTU-3) hybrid at its  $F_6$  generation, with EMS at 0.55% concentration, 17 hours presoaking of seeds in water and 6 hours of the mutagen treatment.

By selfing the tetraploid, some tetraploids and diploids were obtained. The tetraploids have been maintained since eight generations and are breeding true to their induced characters. When compared to their diploid progenitors they exhibited some gigas characters in the plant height, leaf length and breadth, thickness of the midrib of the leaf, and the size of the spikelets. An increase of 25 centimeters in the plant height was observed in the tetraploid on comparison with the diploid plants. The leaf length and breadth measured respectively 43.50 cm and 1.82 cm in the tetraploid whereas in the diploid they were 38.70 cm and 1.25 cm, which is a detectable morphological change. The culms and the leaves were thick and coarse in the tetraploids. The duration of the crop in the tetraploid was 130 days while that in the diploid 100 days and the former was found to be slightly photosensitive. The grains were considerably larger in size with pronounced awning. The length and breadth of the grain were respectively 12.09 mm and 3.17 mm in the 'mutant', which in the diploid they were 7.72 mm and 3.09 mm. The length by breadth (L/B) ratio accordingly in the tetraploid was 3.81 and that in the diploid, 2.49. Concomitant with the increase in size of the grains the 1000-grain weight also increased in the tetraploid (39.53 gm) when compared to the diploid (22.40 gm). The spikelet sterility of the 'mutant' was 70.25% on an average in the eight generations studied. The total number of grains per panicle were slightly reduced in the 'mutant' (diploid-185 grains; tetraploid-151 grains).

At diakinesis the average number of quadrivalents per cell was found to be 6.50 per cell (Table I). The terminalization coefficient at diakinesis was 0.80. Among the quadrivalents, type-17 occurred in high percentage (53.54%), followed by type-11 (19.68%) and type-18 (17.32%). Other types, type-16, type-14 and type-12 occurred at frequencies of 6.29%, 2.75% and 9.39% respectively.

At metaphase-I, the average number of quadrivalents per cell found was 6.30 and that of trivalents and univalents, 0.54 each per cell (Table I). Type-17 and type-11 occurred predominantly in percentages of 63.21 and 21.26 at this stage. The other configurations, type-18, type 12 and type-16 occurred quite infrequently at percentages of 6.60, 4.54 and 4.02 respectively. The terminalization coefficient was 0.94 at this stage. In Fig. 1 a metaphase cell with 3 quadrivalents and 18 bivalents is shown. In both diakinesis and metaphase-I stages ring bivalents were most frequent in occurrence when compared to the rod bivalents (Table I).

At anaphase-I the chromosomes moved to the two poles in a regular way with 24-24 distribution

TABLE I  
Frequency and types of multivalents in the EMS-induced auto-tetraploid of rice

Types of associations		Frequency	
		Diakinesis	Metaphase-I
Bivalents	○	304 (63.55)*	469 (75.90)
	---	146 (46.55)	149 (24.10)
Trivalents	---	0	2 (66.66)
	○-	1	1 (34.34)
Quadrivalents	⊙	136 (53.54)	220 (63.21)
	○ ○	44 (17.32)	23 (6.60)
	----	50 (19.68)	74 (21.26)
	>---	1 (0.39)	16 (4.54)
	-○-	7 (2.75)	1 (0.28)
	○--	16 (6.29)	14 (4.02)

\* Figures in brackets represent percentage.

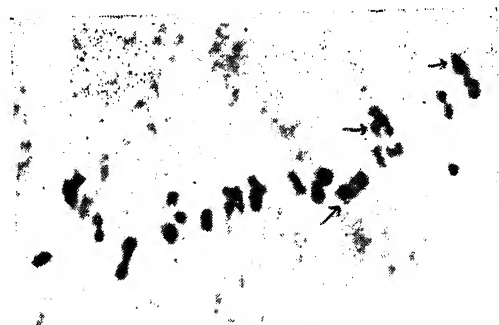


FIG. 1. Photomicrograph of a Metaphase I cell showing 3 quadrivalents (indicated by arrows) and 18 bivalents in the autotetraploid, X 300.

in majority (over 90%) of the cells. Such an observation was reported earlier by many workers<sup>1-6</sup>. The pollen sterility was found to be 65.72% on an average. The pollen diameter of the tetraploid was considerably greater (43.22  $\mu$ ) when compared to its diploid (34.35  $\mu$ ).

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### SOME BIOCHEMICAL STUDIES IN EUROPEAN AND ASIATIC VARIETIES OF CARROT (*DAUCUS CAROTA*)

#### ABSTRACT

Total carotene in fresh carrot, percentage of dry matter and total soluble solids were determined in two selections and ten varieties of European and Asiatic types of carrot. A marked variation in dry matter, total soluble solids and carotene content amongst the varieties was found. European types of carrot were superior to Asiatic types in respect of all the characters included in the study. The bigger size of core and high percentage of moisture was mainly responsible for poor nutritive value of Asiatic types of carrot.

THE significance of vegetables in improving the nutritive quality of foods prompted us to develop vegetable varieties with high nutritive value. Among the vegetables, next to leafy vegetables, carrots contain the largest quantity of precursors of vitamin A. The vitamin value and the high carbohydrate content has been responsible for the increased production and popularity of carrots.

The chemical composition of carrots was reported to vary among the varieties and within the variety depending upon various edaphic factors (Barness, 1936; Werner, 1941). Banga *et al.* (1955) noticed differences in the temperature requirement of carrot varieties for the production of the maximum amount of carotene. This study was undertaken to determine the changes in the chemical composition of a few carrot varieties included in the breeding programme at IARI Vegetable Research Station, Katrain.

#### MATERIALS AND METHODS

Ten varieties and two selections obtained from an intervarietal hybridization were included in the experiment. The samples for analysis were obtained from a replicated trial of carrot being assessed for yield at Katrain during the year 1969-70. Out of the ten varieties included in the experiment, two varieties each of Asiatic and European group were selected for chemical analysis of the core and flesh separately.

A mixed sample of 15 roots was used for each determination. The moisture percentage and total

solids per 100 g of fresh carrot were determined by dehydration. The total soluble solids were calculated using a refractometer (Ruck, 1963). Total carotene was estimated according to the method of Booth (1945). Fresh roots were grated and ground with pure sand in a mortar. The extraction was carried out (in dim light) with about 15 ml of a mixture of 3 parts of petroleum ether (b.p. 40-60° C) and 2 parts acetone, adding a small quantity of anhydrous sodium sulphate. This liquid was then decanted and the extraction repeated until the extract was colourless. The colour intensity of combined extracts was measured in a photo-electric colorimeter, set at 450 nm. The total carotene in the samples was expressed as mg per 100 g of fresh weight.

#### RESULTS AND DISCUSSION

The total carotene content in mg per 100 g of fresh weight, dry matter as per-cent of fresh weight, per cent total soluble solids and total carotene in mg per 5 g of dry matter in carrot varieties are presented in Table I.

Variety Express Osenia, E.C. 41253, Nantes Half Long, Long Imperator and the other European types contained higher percentage of dry matter, whereas Pusa Kesar and Delhi Desi (Asiatic types) were low in dry matter. The maximum quantity of dry matter was 12.6% in Express Osenia and minimum 10.0% in Pusa Kesar. The general trend observed was that the flesh was richer than the core.

The maximum and minimum quantity of total soluble solids was 15.6 in selection 5 and 12.2 in Pusa Kesar. In general, the total soluble solids were greater in European types than in the Asiatic types. It is worth mentioning here that the core alone had more total soluble solids than the flesh.

The results of the analysis for the total carotene in fresh sample (Table I) indicated that European types of carrot were richer in carotene as compared to the Asiatic types. The maximum quantity (4.54 mg) of carotene was observed in selection 1 followed by another selection 2 and the minimum in variety Delhi Desi (2.68 mg). On the other hand when carotene content per 5 g of dry matter were compared, the difference between the maximum and minimum variance was decreased. This was apparently due to the moisture content in the different varieties. Further, it was observed that the core portion of carrot was poorer in carotene content and the varieties which had a large core were poor in carotene content and *vice versa*. Although flesh of Pusa Kesar and Delhi Desi were rich in carotene with 3.65 and 3.53 mg respective

TABLE 1  
Carotene content of different carrot varieties

Variety		Total carotene in mg per 100 g of fresh wt.	Dry matter as percentage of fresh wt.	Percentage of total soluble solids	Total carotene in mg per 5 g of dry matter
Nantes Half Long	European type	4.17	12.1	15.1	1.72
Express Osenia	do.	4.29	12.6	14.5	1.70
Royal Chantenay	do.	3.85	11.6	14.3	1.66
Long Imperator	do.	4.03	12.0	14.9	1.68
Waltham Hicolour	do.	3.68	11.8	15.5	1.56
E.C. 41253	do.	3.74	12.4	15.0	1.51
E.C. 41854	do.	3.28	11.8	15.3	1.39
E.C. 2672	do.	3.25	11.8	15.4	1.38
Selection 5	do.	4.54	11.8	15.6	1.92
Selection 2	do.	4.32	10.9	15.5	1.98
Delhi Desi	Asiatic type	2.68	10.2	13.0	1.31
Pusa Kesar	do.	3.01	10.0	12.2	1.50
Nantes Half Long	(Cores only)	3.24	11.6	14.3	1.40
do.	(Flesh only)	4.19	12.0	12.2	1.74
Selection 5	(Cores only)	3.59	11.0	16.1	1.63
do.	(Flesh only)	4.61	11.2	14.5	2.05
Pusa Kesar	(Cores only)	2.67	9.6	14.0	1.39
do.	(Flesh only)	3.65	10.2	12.0	1.71
Delhi Desi	(Cores only)	2.46	9.6	13.8	1.28
do.	(Flesh only)	3.53	10.0	12.0	1.77

Note : E.C. numbers are Exotic numbers given to the carrot varieties by Plant Introduction Division of I.A.R.I., New Delhi-12.

but the roots had a large core and hence both the varieties were poor in carotene content.

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# NOTE ON EFFECT OF TOP DRESSING OF NITROGEN ON THE PROTEIN CONTENT OF RICE VARIETY I.R. 8

THOUGH several experiments have been conducted on the effect of basal as well as split application of nitrogen on the yield and quality of rice, not much work has been reported in India on the top dressing of nitrogen at heading and its effects on the content of protein in grain and straw. Patnaik *et al.* (1967) observed that higher levels of nitrogen beyond the panicle initiation stage resulted in an increase in the nitrogen content of grain. This effect was more pronounced in straw. Mehrotra *et al.* (1970) indicated that the rate of N uptake irrespective of varieties was 30% upto flag leaf stage while the balance was during the reproductive stage. Ramanujam and Rao (1970) reported that there was a linear increase in the protein content of the grain and in straw with increasing levels of nitrogen from 0 to 180 kg/ha. Latchanna and

seedlings were planted with a basal application of 3 levels of nitrogen, *i.e.*, 40 (N1), 80 (N2) and 120 (N3) kg/ha along with a common dose of 80 kg each of P and K/ha. The top dressing of nitrogen was fixed as a single dose at the rate of 40 kg N/ha at one of the four distinct growth phases of rice, *viz.*, (1) active tillering stage (S1), 47th day, (2) ear primordial initiation stage (S2) 68th day, (3) boot leaf stage (S3) 80th day, (4) at heading stage (S4) 95th day after seeding. The crop was ready for harvest 127 days after seeding. The protein content in grain and straw was determined by estimating the nitrogen (A.O.A.C.) and multiplying with factor 5.95.

## RESULTS AND DISCUSSION

It is evident from Table I that application of nitrogen at boot leaf to heading stage significantly increased the content of protein both in grain and straw, when compared to other stages of application (Table I). This is in agreement with the

TABLE I  
Yield in tonnes/ha and protein content in grain and straw (%)

Levels of N	Grain yield (tonnes/ha)				% Protein (Grain)				% Protein (Straw)			
	N1	N2	N3	Mean	N1	N2	N3	Mean	N1	N2	N3	Mean
Stages of top dressing												
S1	5.86	6.69	6.29	6.28	7.84	7.83	8.11	7.93	3.94	4.14	4.33	4.14
S2	5.76	6.98	6.38	6.37	7.92	7.97	8.16	8.02	3.93	4.28	4.40	4.20
S3	6.35	6.40	6.24	6.33	8.01	8.24	8.37	8.21	4.11	4.50	4.46	4.36
S4	4.91	5.98	6.63	5.84	8.14	8.31	8.33	8.26	4.13	4.52	4.89	4.51
	5.72	6.51	6.39		7.98	8.09	8.24		4.03	4.36	4.52	
C.D. at 5% stage	..			0.403				0.144				0.108
Level	..			0.348				0.126				0.093
Combination	..			0.710				0.252				0.186

Rao (1969) observed that highest increase in protein contents resulted for N application in two splits at planting and at the boot stage. This paper reports the results of a field trial carried out at the Agricultural College and Research Institute, Vellayani, Kerala, during *Kharif* season of 1969.

The experiment was laid out in a randomised block design with four replications with the variety I.R. 8. The soil of the experimental plot was sandy loam in texture with pH 5.4 and contained 0.18% total nitrogen, 9.55 kg/ha available P and 37.6 kg/ha available potash. Twenty-two days old

findings of Murayama *et al.* (1955), and Kik and Hall (1961) who observed that nitrogen supply after heading helped to increase the protein content of grain. Similar results were also reported by Patnaik *et al.* (1967), Mehrotra (1970) and Latchanna and Rao (1969). Application of nitrogen at heading also increased the content of protein in straw (Table I). Matsushima (1967) also reported that if the rice plant is top dressed with nitrogen at heading time, its content in leaf blade is always much higher than in the non-top dressed plants during ripening period. Similar results were also reported by Patnaik *et al.* (1967). The higher

content of protein in grain and straw for the application of nitrogen during generative growth phase may be due to the better absorption of the applied nitrogen and increased utilization during that stage.

The protein content both in the grain and in straw increased significantly with increase in the level of basal nitrogen (Table 1). However, there does not seem to exist a significant association between grain yield and protein content.

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## CYTOLOGICAL EFFECTS OF THE INSECTICIDE "SEVIN" ON THE MEIOTIC CELLS OF *POECILO CERUS PICTUS*

"SEVIN" (1-naphthyl-*n*-methyl carbamate) belonging to the carbamate group of insecticides having a lower toxicity to mammals and a high specificity of action on insects<sup>1</sup> has been employed against cotton pests<sup>2</sup>, different crops including vegetables<sup>3</sup> and even as a domestic pesticide. Its cytological studies on mitotic<sup>4</sup> and meiotic<sup>5,6</sup> cells of plants indicated the production of sticky diakinesis stages, fragments, bridges, lagging and unequal separation of chromosomes. No increase in the chromosome rearrangements was noticed after treatment in salivary chromosomes of *Drosophila melanogaster*<sup>8</sup> or in those of rat bone marrow cell<sup>7</sup>. In the former while its mutagenic action was described by Haque<sup>3</sup>, a lack of either an increase in the mutation frequency or a reduction in the fertility was reported by Brzeskij<sup>8</sup>. The cytological effects of this insecticide observed on the meiotic cells of the grass hopper, *Poecilocus pictus*, used as the test animal and the possible hazards caused by this environmental contaminant have been reported in this paper.

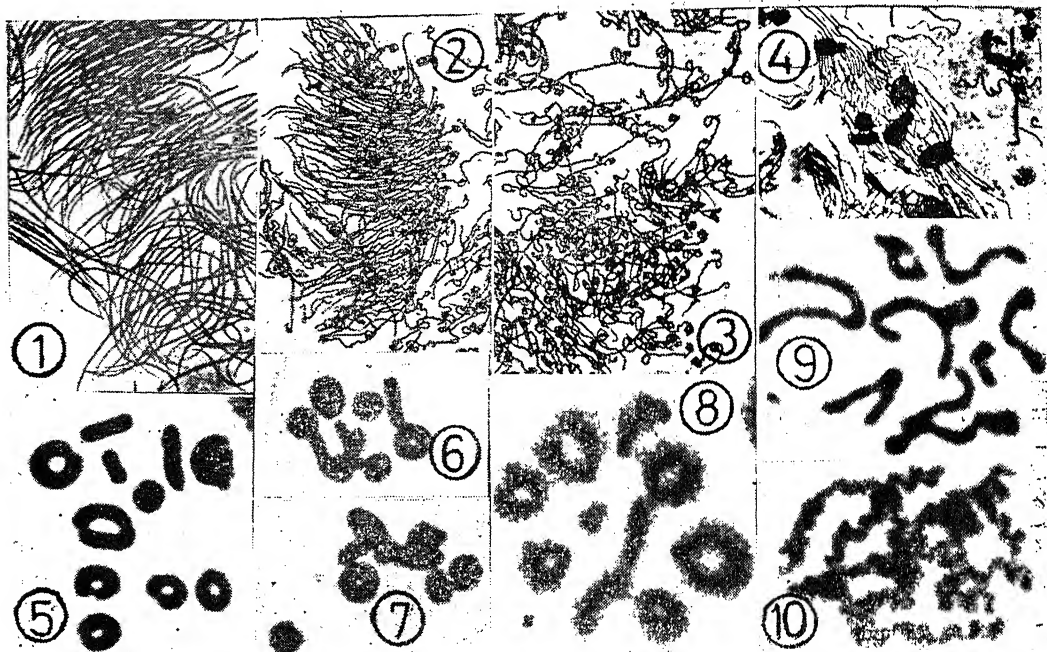
**Materials and Methods.**—0.25 ml of supernatant Sevin (99.7% ; solubility, 0.1% ; Union Carbide Corporation) in sterile distilled water, was injected laterally between the third and fourth abdominal segments of the male grass hoppers. The control animals received an identical volume of sterile distilled water. The testes of the animals maintained at room temperature were dissected out at the end of 1, 4, 8, 12, 16, 20, 24 and 48 hours, treated with 0.9% sodium citrate, fixed in acetic alcohol (1 : 3) and processed as haematoxylin squashes as described elsewhere<sup>9,10</sup>.

**Observations.**—The animals treated for forty-eight hours were more sluggish than others which might be due to the action of Sevin behaving as a nerve poison like organo-phosphorus compounds. The changes in the morphology of spermatozoa observed were clustering and spiralization of parts of spermatozoa and an accentuation of the effect with an increase in the time treatment (Figs. 1, 2 and 3). Instances of breakages in spermatozoa were also occasionally seen (Fig. 4).

In comparison with the controls at diakinesis (Fig. 5) there was a tendency for over contraction of the bivalents (Fig. 6) and towards clumping and stickiness (Fig. 7). The structural chromosomal aberrations, breakages, lagging and other changes were not observed. The treatment with the insecticide had not only led to the elucidation of the lamp brush structure of chromosomes from early and late diakinesis (Fig. 8) but also had

revealed the chromosome coiling at anaphase (Fig. 10; compare with Fig. 9).

The radical alteration in the morphology of same spermatozoa in treated specimens (compare Figs.



FIGS. 1-10. Fig. 1. Sperms from control specimen,  $\times$  ca. 250. Figs. 2 and 3. Clustering and coiling of spermatozoa treated with Sevin for 24 and 48 hours respectively,  $\times$  ca. 250. Fig. 4. Spermatozoa showing breakages, 4 hr treatment,  $\times$  ca. 250. Fig. 5. Diakinesis from control specimen,  $\times$  ca. 800. Figs. 6 and 7. Diakinesis. Note the contraction of chromosomes (Fig. 6) and tendency for stickiness (Fig. 7). 20 hr treatment,  $\times$  ca. 800. Fig. 8. Lamp brush nature of the chromosomes in late diakinesis, 4 hr treatment,  $\times$  ca. 800. Fig. 9. A single group of chromosomes. Anaphase I. Control,  $\times$  ca. 900. Fig. 10. Chromosome coiling. Group of chromosomes at anaphase I, 48 hr treatment,  $\times$  ca. 1,300.

**Discussion.**—It has been known that some agents which induce chromosome breaks in plants may not do so in animal chromosomes and *vice versa*<sup>11</sup>. In the present investigation neither lagging nor breaks and fragments nor other structural chromosomal rearrangements were visualized. The only common feature between the effects of plant and animal chromosomes observed was the tendency for contraction leading to clumping and stickiness. This is not unusual since chromosome contractions<sup>12,13</sup> have been described to be brought about by carbamates which are not insecticides and such phenomena produced by chemicals have been discussed by Anderson<sup>14</sup>. As compared to the controls (Figs. 5 and 9) the accentuation of the lamp brush nature (Fig. 8) and the chromosome coiling (Fig. 10) seen after the treatment was not surprising since one of the methods of revealing the chromosome structure has been the prior exposure of the material to one or the other of a variety of physical and chemical agencies<sup>15-20</sup>.

2 and 3 with 1) affecting their motility and viability might be regarded as one of the abnormalities since they would not be competent enough to participate in fertilization. These results and the contraction and stickiness of chromosomes observed in some cells impairing the normal meiosis indicate the possible hazards of the accidental ingestion of Sevin along with food stuffs by domestic animals and humans.

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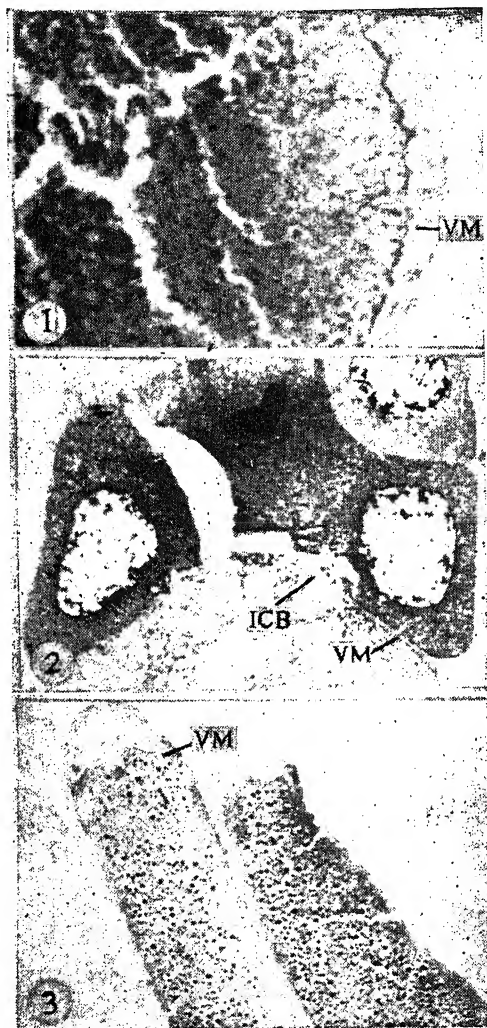
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#### FORMATION OF VITELLINE MEMBRANE IN THE EGG CHAMBERS OF *CHRYSOMYIA MEGACEPHALA* (FABR.)

THE formation of the vitelline membrane which forms a flexible waterproof covering about the oocyte<sup>1</sup> has been described for a few dipteran insects such as *Anopheles maculipennis*<sup>2</sup>, *Drosophila melanogaster*<sup>3-4</sup>, *Dacus tryoni*<sup>5</sup> and *Musca domestica*<sup>6</sup>. In general, these investigations have shown that this membrane is secreted by follicle cells. On the other hand, it is reported for *Culex fatigans* that the oocyte is involved in the formation of this membrane<sup>7</sup>. In view of these conflicting findings it was thought worthwhile to study the formation of vitelline membrane in another dipteran, *Chrysomya megacephala*.

Of the seven distinct stages of ovarian growth described for *C. megacephala*<sup>8</sup>, stage IV is marked by the appearance of vitelline membrane between the follicle cells and the oocyte as well as between the oocyte and the border cells. It appears transparent without any reaction with haematoxylin (Fig. 1). This

membrane is practically absent from the site of the intercellular bridges and this results in free communication of the cytoplasm of the nurse cells with that of the oocyte (Fig. 2). During stage V of follicle development this membrane is found completely enveloping the oocyte (Fig. 3) as in *Drosophila melanogaster*<sup>3-4</sup>, *Dacus tryoni*<sup>5</sup> and *Musca domestica*<sup>6</sup>.



FIGS. 1-3. Fig. 1. Section of a portion of stage IV egg chamber showing vitelline membrane. Fig. 2. Section of a portion of stage IV egg chamber showing the absence of this membrane at the site of intercellular bridges. Fig. 3. Section of a stage V egg chamber showing this membrane completely enveloping the oocyte. [ICB—Inter cellular bridge; VM—Vitelline membrane].

The fact that the vitelline membrane in *C. megacephala* is not initially formed at the sites of inter-

cellular bridges rules out the possibility of proximal nurse cells being involved in the elaboration of this membrane. This inference is consistent with that of King<sup>1</sup> made for *Drosophila melanogaster*.

Nicholson<sup>2</sup> has reported for *Anopheles maculipennis* that the vitelline membrane is secreted between the oocyte and the follicle cells and also between the nurse cells and the follicle cells and concluded that the membrane is secreted by follicle cells. Observations of Gill<sup>4</sup> on the follicles of fs(3)<sup>339a</sup> ovaries of *Drosophila melanogaster* also emphasize the role of columnar follicle cells and border cells in the formation of vitelline membrane. On the other hand, Nath<sup>7</sup> believes that the vitelline membrane is secreted by the oocyte itself. His view is based upon the fact of the formation of the membrane fully between the nurse cells and the oocyte.

Using electron microscopy King and Koch<sup>9</sup>, in their study on *Drosophila melanogaster*, have suggested that the columnar follicle cells, after reaching a certain stage of growth, manufacture much of the precursor materials, which find their way to the intercellular spaces between the follicle cells and the oocyte and eventually fuse to form the vitelline membrane. But in the present study, using light microscopy, though such tiny precursor vitelline bodies could not be detected, the formation of a very thin membrane between the follicle cells and the oocyte as well as between the follicle cells and the border cells was noticed during stage IV of the follicle. Later, this membrane had become thicker to appear distinctly in stage V of the follicle. The occurrence of this membrane as a thin structure during the early stage of the follicle in *C. megacephala* may prompt one to suggest that this structure is identical to the precursor vitelline bodies reported by King and Koch<sup>9</sup> but such an inference warrants further examination especially under electron microscope.

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#### AMINO ACID INCORPORATION BY PM-SUPERNATANT FROM BEAN AXES

PROTEIN synthesis is an extremely co-ordinated and multi-faceted work of small particles, the ribosomes. Having established that protein synthesis is an essential step in the activation of the quiescent seed<sup>1</sup> to obtain the capacity to synthesise molecules necessary for growth, that process of protein synthesis proves its principal necessity for morphogenesis. The machinery of protein synthesis is rather complex in eukaryotes than in prokaryotes. To test the capacity of cell-free amino acid into protein, the system should normally consists of cations K and Mg, ATP and its generating system, GTP, sulphydryl compound, ribosomes (microsomes and supernatant enzyme fraction), tRNA and the presence of mRNA<sup>2</sup>.

In order to have an idea of the efficacy of two systems namely mungbean (*Phaseolus aureus*) and runner bean (*Phaseolus coccineus*) axes, for amino acid incorporation *in vitro*, experiments were conducted with a fraction designated as PM-supernatant (post-mitochondrial supernatant). Richardson *et al.*<sup>3</sup> reported that cell-free system with PM-supernatant closely resembled the intact cell in polysomal aggregation and protein synthesising capacity. The PM-supernatant system has been used only to limited extent and it is proposed to test the efficiency of this system with plant material, the embryonic axes of mungbean and runner bean.

**Materials and Methods.**—The mungbean axes from ungerminated (0 hr) or germinated (4 and 10 hr seeds and from ungerminated (0 hr) and 12 hr germinated (12 hr) runner bean axes were used. The tissue is homogenised in 2 ml buffer containing *tris*-HCl (pH 7.8), 0.2 M; magnesium acetate, 0.02 M; KCl, 0.12 M and mercaptoethanol, 0.03 M. The homogenate was centrifuged at 13,600 g for 20 min and 1.5 ml of the clear supernatant was passed through Sephadex-G. 25 (30 × 1.5 cm) column previously equilibrated with the same buffer. The fractions were measured at 260 nm in a Beckman-DB spectrophotometer. The fractions having high optical density were pooled and used for the assay of amino acid incorporation *in vitro*.

The standard assay mixture contained in  $\mu$ mole quantities the following: *tris*-HCl, (pH 7.8), 100;

magnesium acetate, 10; KCl, 60; mercaptoethanol, 15; ATP, 3; GTP, 0.1; phosphoenol pyruvate, 5; with pyruvate kinase, 20  $\mu$ g; 19 unlabelled amino acids, 2  $\mu$ g in each and  $^{14}$ C-leucine, 0.2  $\mu$ c (331  $\mu$ c per  $\mu$ mole). The ribosomal particles and supernatant fraction (enzymes, factors, tRNA, etc.) were supplied in the form of 0.5 ml of Sephadex-passed sample in a total volume of one ml.

The incorporation assays were performed at 37°C for 30 min. The reaction was terminated by adding 1 ml of 40  $\mu$ M cold leucine in 20% trichloroacetic acid (TCA), and allowed to stand in cold for at least 30 min. The precipitate was centrifuged, incubated for 20 min at 80°C with 3 ml of 10% TCA. The contents were cooled and filtered on Whatman GF/C glass fibre filters; washed twice with 5% TCA (0°C) and once 75% ethanol (0°C). After drying, they were counted with 5 ml toluene-PPO scintillation mixture in a Packard liquid scintillation spectrometer.

In addition to the normal incorporation assay, effect of cycloheximide, 20  $\mu$ g; chloramphenicol, 50  $\mu$ g and RNase, 50  $\mu$ g per assay were also tried.

**Results and Discussion.**—The data presented in Table I brings out a definite relationship of protein synthesising capacity at different hours of early germination. The system from ungerminated axes has practically no capacity to incorporate amino acids into protein. This is suggested by the fact that RNase which destroys mRNA and thereby preventing protein synthesis does not have any effect on the ungerminated system. By the addition of chloramphenicol and cycloheximide to the assay mixture, no alteration in the rate of amino acid incorporation is found.

TABLE I

Cell-free protein synthesising capacity of ungerminated and germinated axes of mungbean and runner bean

(incorporation of  $^{14}$ C-leucine into protein)

Conditions	mungbean			runner bean	
	hr			hr	
	0	4	10	0	12
	cpm per unit				
Complete	430	854	10,533	641	3,690
+ RNase	374	133	315	528	302
+ Chloramphenicol	489	424	11,552	558	3,716
+ Cycloheximide	367	436	11,288	588	3,626

One unit: OD<sub>260</sub> nm: 10.0 (PM—supernatant).

RNase and chloramphenicol, 50  $\mu$ g/ml; cycloheximide 20  $\mu$ g/ml.

The cell-free system from 4 hr germinated mungbean axes has double the capacity of the 0 hr system for incorporation of  $^{14}$ C-leucine into protein. RNase decreases the incorporation by about 85%. The protein synthesis inhibitors, chloramphenicol and cycloheximide inhibit the reaction by about 50%. The 10 hr germinated system is capable of a higher amino acid incorporation as compared to 0 and 4 hr systems. The synthesis is considerably reduced by RNase. But chloramphenicol and cycloheximide have no effect on the incorporation of amino acids into protein.

Similar experiments were carried out with dry and 12 hr germinated runner bean axes. The ungerminated system has only a very low incorporating capacity and the values obtained with the addition of RNase, chloramphenicol and cycloheximide do not differ much from the normal 'complete' system. However, the incorporating capacity increases by about 6-fold in the 12 hr system. The system is highly sensitive to RNase which showed an inhibition of about 90%. The incorporation is resistant to the action of chloramphenicol and cycloheximide.

Cell-free protein synthesis with isolated cytoplasmic ribosomes and purified supernatant fraction is rather a long analytical procedure. The method applied in the present study is comparatively crude, but shows an estimate of the systems that are to be compared, before making further analysis with purified ribosomal and supernatant fractions. The trend in values is similar to that of the purified system<sup>1</sup>. Richardson *et al.*<sup>3</sup> also reported that PM-supernatant system has a greater *in vitro* amino acid incorporation capacity than other *in vitro* amino acid incorporation systems. This method may be regarded as a prelude, before taking up analysis with purified ribosomal fractions. The ribosomes and mRNA, enzyme fractions, necessary factors, tRNA, etc., are all present in the purified PM-supernatant itself, which is simple to prepare. In general, plant systems are rather complex and as such, easier and faster methods will be able to have a fundamental idea on the efficacy of the system in question before making an effort with the purification method. The systems work well and the results are reproducible with the present method described.

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### MICROSPOROGENESIS IN *STELLARIA AQUATICA* (SCOP.)

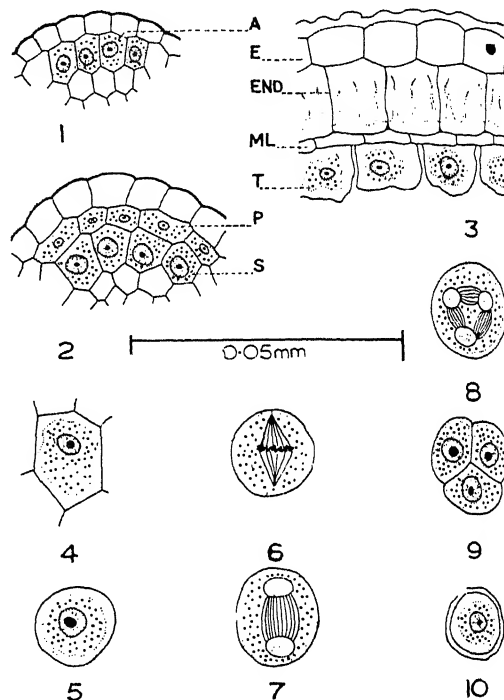
THE embryology of Caryophyllaceae has received considerable attention of earlier workers (see Davis)<sup>1</sup>. Joshi<sup>2</sup> and Pal<sup>3</sup> contributed to the microsporogenesis of *Stellaria media* and *Polycarpon leofflangiae*. The present note accounts for the microsporogenesis in *Stellaria aquatica*.

A mature anther is four lobed in transverse section. The wall consists of an outer layer of epidermis, single layered endothecium, a single middle layer and an innermost tapetum. The cells of epidermis are cuticularised. The endothecium develops characteristic fibrous thickening. Middle layer consists of rectangular to irregular cells. The cells of the glandular tapetum possess dense contents and prominent nuclei.

A cross-section of a very young anther shows a homogeneous mass of cells surrounded by epidermis. It becomes slightly four lobed and hypodermal archesporial cells differentiate in each of the four lobes by their large size, dense cytoplasm and conspicuous nuclei (Fig. 1). In each lobe there may be four to five archesporial cells (Fig. 1). The archesporial cells divide periclinally to form an outer primary parietal layer and an inner primary sporogenous layer (Fig. 2). The primary parietal layer divides repeatedly periclinally and anticlinally and the outer components form an endothecium and a single middle layer. From inner components tapetum is differentiated (Fig. 3). Thus the development of anther is of a dicot type (Davis, 1966).

The primary sporogenous cells divide resulting into a mass of sporogenous tissue of which most of the cells may act as spore mother cells while the rest degenerate. The cells undergo reduction division and each simultaneously forms four microspores (Figs. 4-9). Tetrahedral microspores were observed (Fig. 9). Thus microsporogenesis is simultaneous type as has been reported by Joshi (1936) in *Stellaria media*. The microspores separate off from each other and become spherical (Fig. 10). A young microspore has a large nucleus and dense cytoplasm. Soon it enlarges considerably and the exine develops ornamentations.

During maturation of the anther the epidermal cells shrivel and middle layer disintegrates. The tapetum persists until meiosis and the separation of microspores but ultimately it is completely absorbed. The anther wall at the time of dehiscence comprises a degenerated epidermis and an endothelial layer. The pollen grains are two celled at the time of shedding. The dehiscence is by a longitudinal slit.



FIGS. 1-10. Figs. 1-3. Development of anther wall layers and stages in microsporogenesis. Figs 4-9. Various stages in development of the pollen grains. Fig. 10. Single celled pollen grain.

A—Archesporial cells; E—Epidermis; END—Endothecium; ML—Middle layer; P—Primary parietal layer; S—Primary sporogenous layer; T—Tapetum.

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For a long time, perhaps, has been definitely formulated the idea that the vascular bundles are more conservative than its external form. To quote Henslow:



"every organ can be met with in any stage of degeneration till it has completely vanished and even when all visible trace is wanting, the vascular cord belonging to it may in some cases still detected. Last of all this vanishes as well". This idea received support from various workers (see Puri, 1951; Eames, 1961)<sup>2,3</sup>. More recently this view has been strongly advocated by Moseley<sup>4</sup> who asserted that "The vascular system is *nearly always* more conservative than the organs it supplies".

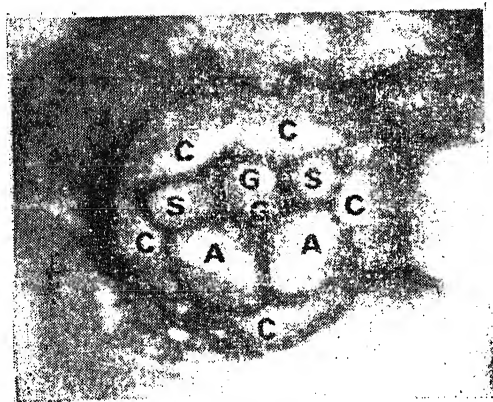


FIG. 1

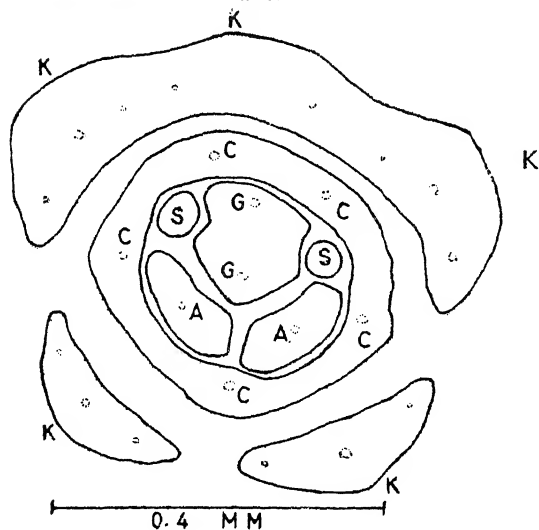


FIG. 2

FIGS. 1 and 2. *Justicia gendarussa*. Fig. 1. Photograph from the dissection of a floral bud after inception of gynoecial primordia. Sepals have been removed to exhibit the primordia of subsequent whorls of appendages. Note primordia of posterior pair of rudimentary stamens (S),  $\times 120$ . Fig. 2. Cross-section of a floral bud of the same stage as in Fig. 1. While the primordia of anterior pair of fertile stamens (A) show one procambial strand each, those of the posterior pair of rudimentary stamens (S) show a complete lack of vascular tissue.

A—primordium of fertile stamen; C—petal primordium; G—gynoecial primordium; K—sepal primordium; S—primordium of rudimentary stamen.

Arber<sup>5,6</sup> in her earlier writings also supported this view but later she revised her opinion and became a strong opponent of it. She wrote<sup>7</sup> that a "rudimentary form is found to correspond to a vascular system which is equally, or even more, rudimentary; indeed an organ which retain some trace of its external form, may yet show a complete lack of vascular tissue. It thus becomes clear that we have no alternative but to discard the doctrine of conservatism of the vascular bundle." There are several other cases on record where floral organs exist (in various stages of degeneration) but without any vascular tissue<sup>8-13</sup>. Recently Carlquist<sup>14</sup> also ridiculed the doctrine of conservatism of vascular bundles. He wrote that "vascular bundles are formed with relation to actual organs and not non-existent ones".

During the course of study of the floral development of *Justicia gendarussa* Burm. (Acanthaceae) we have observed primordia for the posterior pair of missing stamens (Fig. 1) (only two stamens forming anterior pair are reported in this species in published literature). The growth of these primordia is arrested in early stages of development, however, in a few cases they develop into rudimentary stamens. The floral buds with the primordia for posterior pair of stamens were also sectioned to study the procambial development. They showed that while the primordia of fertile stamens (anterior pair) receive one procambial strand each, those of the rudimentary stamens were without any vascular supply (Fig. 2). Thus in *Justicia* where the anterior pair of stamens are in a state of reduction, show a complete lack of vascular tissue. In the face of such instances we have to discard the expression of "conservatism of vascular bundles".

School of Plant Morphology,  
Meerut College,  
Meerut, January 18, 1974.

V. SINGH.  
D. K. JAIN.

\* Research contribution No. 120 from the School of Plant Morphology Meerut College, Meerut.

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## SHORT SCIENTIFIC NOTES

### A New Host for *Catenulaster batistae* Agarwal and Sharma

During the study of the fungus flora of Jabalpur the author encountered a fungus on immature fruits of *Dalbergia sissoo* Roxb. which forms epiphyllous, black, punctiform, superficial scattered bodies easily detachable with the help of needle. The diagnostic characters of the fungus is as follows :

Free mycelium lacking, pycnostroma superficial, epiphyllous, orbicular, scutellate, brown, glabrous, pseudostiolate, wall prosenchymatic, upto  $6.5\ \mu$  thick, subhyaline at margin,  $80-160\ \mu$  in diam., av.  $120\ \mu$ ; conidiophores indistinct; pycnidiospores elliptical to bacillar, hyaline, catenulate, sessile,  $2.2-4.2 \times 2.2-3.2\ \mu$ , av.  $3.5 \times 2.8$ .

Except few minor differences especially in measurements of pycnidia the species is very close to *C. batistae* Agarwal and Sharma, the only species represents the genus *Catenulaster* Batista and Costa in India<sup>1</sup>. This is a new host record from India.

On pods of *Dalbergia sissoo* Roxb. (Papilionaceae), Botanical garden, Govt. Sci. College, Jabalpur, May 1969, Leg. N. D. Sharma. The specimen has been deposited in the Herbarium, I.M.I., Kew, No. 140910.

The author expresses his grateful thanks to Dr. G. P. Agarwal, Head, Department of Post-Graduate Studies and Research in Botany, University of Jabalpur, for encouragement. Thanks are also due to Mr. A. Johnston, Director and Mr. Sutton of the Commonwealth Mycological Institute, Kew, for help in the identification of the species.

Dept. of Post-Graduate Studies and Res. in Botany,  
University of Jabalpur,  
January 23, 1974.

N. D. SHARMA.\*

\* Present address : Department of Plant Pathology, J.N. Agric. University, Jabalpur-4.

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### Isolation of *Verticillium dahliae*

H. N. Gaur and H. C. Dube (Department of Botany, University of Udaipur, Udaipur-313001) report the isolation of *Verticillium dahliae* (with its characteristic verticillate conidiophore and microsclerotia *in vitro*) from wilted cotton plants from

Banswara District, about 120 Km North of Udaipur, Rajasthan. The isolate was successfully transmitted by injection of conidial suspension of the pathogen into 12-day old cotton plants (*Gossypium hirsutum* local variety). Characteristic yellowing symptoms resulted from the inoculation and re-isolation of the fungus from petioles and roots and proved Koch's postulates. [Isaac, I. (1949), *Ann. appl. Biol.*, 32, 137-157; Natarajan, M. K., Sivaprakasam, K. and Ramakrishnan, K. (1968), *Madras agric. J.*, 55, 455; Isaac, I., Pandian, T. T., Saraswathi-Devi, L. and Dube, H. C. (1972), *Trans. Br. mycol. Soc.*, 59, 313].

### On the Occurrence of *Glossiphonia heteroclita* (Linneus) (Annelida: Hirudinea) from Rajasthan, India

While examining the leech collection of Rajasthan we came across many of specimens of leech collected from the District of Nagaur of Rajasthan by Dr. B. Biswas, during September, 1960.

The following observations are based partly on the study of the living forms and partly on the study of fixed specimens. The body is ovate acuminate, flattened, smooth, transparent, and of clear amber-yellow colour. The three pairs of eyes vary to some extent in position, but usually lie in ring 5, 7, 8 respectively. Size :  $12 \times 5\ \text{mm}$  at normal condition.

*Material*.—9exs. from tanks of Rol-qazian, Didia, and Singar of District Nagaur, Rajasthan.

A review of the literature reveals that this species is a rare one, and the only known record is by the original author (Blanchard) of the species from Europe and North America.

Harding and Moore in the *Fauna of British India* give the area of the distribution of this species as Burma.

It seems that this particular species is not recorded from the Indian sub-continent. Thus the present note is intended to place on record the actual occurrence of the Species of *Glossiphonia heteroclita* (Linn.) from the Indian sub-continent.

Asst. Zoologist, MAHESH CHANDRA.  
Zoological Survey of India, R. N. MUKHERJEE.  
Solon, October 11, 1973.

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## REVIEWS AND NOTICES OF BOOKS

**A Geometric Introduction to Topology.** By C. T. C. Wall. (Addison-Wesley Pub. Co., Inc., Reading, Massachusetts-01867), 1972. Pp. vi + 168. Price \$7.95.

In this recent addition to topological literature, the reader is taken through the terrain of topology and is pointed out the geometrical features of interest. It is of particular use to one just getting interested in topology, since the pre-requisites are kept down to a minimum.

The book is divided into four parts. Part 0 disposes off the pre-requisites. Here the basic topological notions regarding  $n$ -dimensional Euclidean spaces  $\mathbb{R}^n$  are dealt with using illustrative examples from geometry. A brief discussion of Abelian groups is also found here.

Part 1 completes the spade work necessary for the rest of the book. Notions of connectedness are discussed and homotopy is introduced. Three groups associated with a topological space  $X$  are introduced—the groups  $H^0(X)$ ,  $H^1(X)$  and  $H_0(X)$ .

The path and homotopy lifting properties are proved for the relevant cases. Using the notion of degree of a map of  $S^1$  (the unit sphere in  $\mathbb{R}^2$ ) into itself, the celebrated 'Fundamental Theorem of Algebra' and the 'Brouwer's Fixed Point Theorem' are proved. Some algebraic tools for the computation of the groups  $H^1(X)$  are given.

Part 2 deals with what the author calls 'the high point of this text'—'The Alexander Duality Theorem'. This is proved in several short stages. It is followed by an extremely short and elegant proof of the famous 'Jordan Curve Theorem'. Connections with other branches like projective geometry are mentioned in the last chapter of this part.

Part 3 opens with two alternative proofs of the 'Jordan Curve Theorem' and its consequences.

Next, the first homology group  $H_1(X)$  is introduced and a second duality map is discussed briefly.

The concluding chapter deals with integration. Differential forms and the exterior derivative are introduced informally and some connections between groups arising from them and the homology groups of open subsets of  $\mathbb{R}^2$  are mentioned. Extensions to three dimensions are indicated.

This book will not serve as a text for a serious course on algebraic topology, nor is it intended for this purpose. Features like the fundamental group, Singular Homology theory, etc., are excluded from treatment. The author, in his preface, explains his reasons for this unconventional (yet interesting) approach.

S. KESAVAN.

### Books Received

*Invariant Imbedding and Its Applications to Ordinary Differential Equations and Introduction.* By Melvin R. Scott. (Addison-Wesley, Inc., Reading, Mass. 01867), 1973. Pp. xiii + 215. Price: Cloth binding, \$19.50; Paper binding, \$11.50.

*Proceedings of the Symposium on Living Resources of the Seas Around India.* (Central Marine Fisheries Research Institute, Post Box No. 1912, Cochin-682018), 1973. Pp. v + 748. Price Rs. 80.00.

*Hadron Physics at Very High Energies.* By David Horn, Fredrik Zachariasen. (Addison-Wesley Pub. Co., Reading Mass. 01867), 1973. Pp. xvii + 378. Price: Cloth binding, \$17.50; Paper binding, \$9.50.

*Introduction to Atomic and Nuclear Physics* (5th Edition). By Henry Semat, John R. Albright. (Chapman and Hall, Ltd., 11 New Fetterlane, London EC4 P. 4CE), 1939. Pp. xv + 712. Price £4.20.

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Shilpi-2-SYS 4/72

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AND

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# ABSTRACT

In addition to the conventional dihedral angle associated with a sequence of four atoms, a new type of dihedral angle, also associated with four atoms P, Q, R, S, is defined as the angle between the planes containing P, R, S and Q, R, S, when P and Q are both attached to the same atom R. The notation  $\xi$  (P, Q; RS) is adopted for this angle. It is pointed out that the conformational energy of a biomolecule may also contain contributions due to the distortions produced by the changes in rotation angles of the type defined here.

THE use of dihedral angles for the specification of the backbone conformation of neighbouring peptide units was first enunciated by Ramachandran, Sasisekharan and Ramakrishnan<sup>1</sup> in 1963. These angles have been standardized in the rules formulated by the International Union of Pure and Applied Biophysics<sup>2</sup>. According to these rules, a general dihedral angle associated with four atoms A, B, C, D as in Fig. 1 (a) is given by the angle between the two planes containing the atoms A, B, C and B, C, D respectively, the sign of the angle being considered to be positive if the latter plane is obtained from the former by a clockwise direction looking from B to C. The *cis*-convention is used for  $\theta = 0$ , i.e., the torsion angle  $\theta$  is taken to be zero when the atoms A and D in the sequence A—B—C—D are *cis* to each other.

According to the international rules, the terms dihedral angle, rotation angle, torsion angle, are all applicable for the angle  $\theta$  as defined above. It is the purpose of this note to indicate that another type of dihedral angle can be defined for a set of four atoms P, Q, R, S, which are connected by bonds as in Fig. 1(b), which is different from the connectivity adopted in Fig. 1(a). Here the two atoms P and Q are both attached to the atom R which in its turn is connected to an atom S by the bond RS. The relevant angle  $\theta$  is indicated in the figure both in magnitude and sign, and it is the angle between the planes containing P, R, S and Q, R, S respectively. We may define  $\theta$  to be zero in the imaginary situation (as far as

normal organic molecules are concerned) when Q lies in the plane PRS and on the same side of RS as P (P and Q are *cis*) and its sense to be positive if the angle of rotation about RS, looking from R to S, is clockwise for going from the plane containing P to the plane containing Q.

The definitions of the two types of dihedral angles are particularly clear from the Newman projections shown in Figs. 2 (a) and 2 (b). In order to distinguish between the two types, it is suggested that the former may be called 'torsion angle' (symbol  $\chi$ ) and the latter 'rotation angle' (symbol  $\xi$ ). Where the precise nature of the angle is not essential to be defined, both of them could be denoted by the term 'dihedral angle' (symbol  $\theta$ ). If the atoms concerned have to be specified, the detailed form of the torsion angle may be denoted by symbols of the form  $\chi$  (A—B—C—D),  $\chi$  (A, B, C, D) or even briefly as  $\chi$  (A, D). (The symbol  $\chi$  agrees with that used for side-chain torsion angles in the IUPAB rules<sup>2</sup>). Under similar circumstances, the rotation angle may be denoted by  $\xi$  (P, Q; RS) or simply  $\xi$  (P, Q) when the axis of rotation is not in doubt.

The use of the two types of dihedral angles for the specification of the full three-dimensional structure of a molecule may be illustrated by the example of ethane, whose atoms are numbered as in Fig. 3 (a), and shown in a Newman projection in Fig. 3 (b), corresponding to the staggered conformation. Apart from the seven bond lengths and the six bond angles (three at C<sub>1</sub> and three at C<sub>2</sub>) that are normally defined, it is necessary to define five dihedral angles to obtain the total of 18 internal parameters that are required for specifying the conformation of the molecule.

\* Contribution No. 45 from the Molecular Biophysics Unit.

\*\* Address to which reprint requests are to be sent.

Using the definitions mentioned above, these are most conveniently represented by the following set:

$$\begin{aligned} \chi(3, 1, 2, 6) \\ \xi(3, 4; 12) \quad \xi(3, 5; 12) \\ \xi(6, 7; 21) \quad \xi(6, 8; 21) \end{aligned}$$

In this set, only one torsion angle is adopted for the rotation about the bond  $C_1-C_2$ , which is the only bond in this molecule that does not have a terminal atom at either end. In the same way, it can be shown that, in a general molecule, having  $N$  atoms, the number of bonds ( $b$ 's) necessary to be included in the list of parameters is  $(N-1)$ , which is readily seen by converting the graph representing the connectivity of the atoms in the molecule into a 'tree'. (See for example the case of cyclohexane shown in Fig. 4). So also the minimum number of bond angles ( $\tau$ 's) required is  $(N-2)$ . As regards the remaining  $(N-3)$  dihedral angles, the minimum number of torsion angles ( $\chi$ 's) that is required is  $n$ , where  $n$  is the number of non-terminal bonds in the tree. The remaining parameters required can all be specified as rotation angles ( $\xi$ 's), whose number is obviously  $(N-n-3)$ .

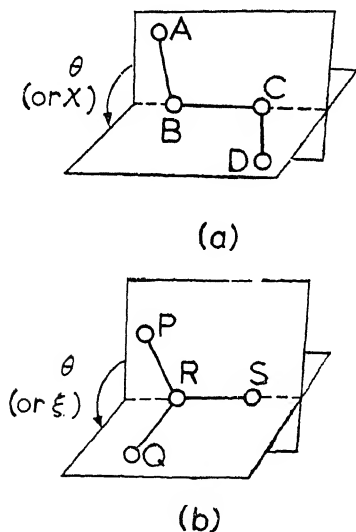


FIG. 1. Diagram showing the definitions of the two types of dihedral angles; (a) Torsion angle  $\chi$  and (b) Rotation angle  $\xi$ .

The above list of internal parameters are those that are required for the complete specification of the three-dimensional structure of the molecule concerned. Actually many more angles can be defined in the molecule—for example  $\chi(C_1, C_2, C_3, C_4)$ ,  $\chi(C_2, C_3, C_4, C_5)$ , or  $\xi(H_{11}, H_{12}; C_3C_4)$  in Fig. 4—which may be required for calculating the total energy of the molecule. The details of these aspects as well as the proof of

the statements made in the previous paragraph will be discussed in a separate communication.

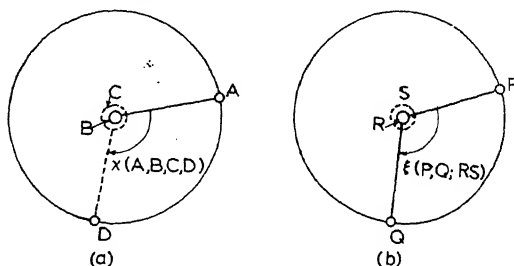


FIG. 2. Newman projections of the conformation of the atoms used in the definition of (a)  $\chi$  (A, B, C, D) and (b)  $\xi$  (P, Q; RS).

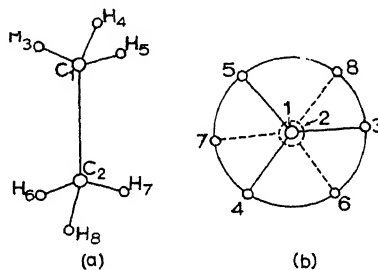


FIG. 3. (a) Perspective diagram of the ethane molecule,  $C_2H_6$ . (b) Newman projection of the atoms contained in the molecule.

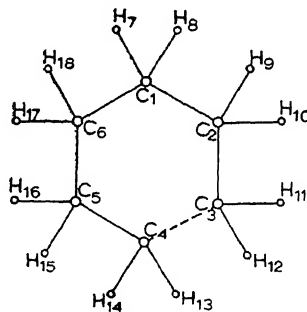


FIG. 4. Schematic diagram of the atoms  $C_1$  to  $C_6$  and  $H_1$  to  $H_{18}$  of the molecule cyclohexane, showing their connectivities. The bond between  $C_1$  and  $C_4$ , shown by dotted lines, has been removed to convert the corresponding graph into a 'tree'.

The fact that the new type of dihedral angle ( $\xi$ ) is required in practical examples is illustrated by the case of the non-planar peptide unit<sup>3</sup> proposed from the author's laboratory. Denoting the atoms in a single peptide unit by the symbols  $C_1, C', O, N, H, C_2$ , the angle  $\theta_N$ , defined therein to indicate the non-planarity of the three bonds meeting at the nitrogen atom, can be described in terms of the rotation angle  $\xi(C_2, H; NC')$ . (See Ref. 3 for diagrams). The relation between  $\xi$  and  $\theta_N$  is

$$\theta_N = 180^\circ - \xi$$

where  $\xi = 180^\circ$  defines the planarity of the peptide unit at the nitrogen atom. This illustrates the great importance of the newly defined dihedral angle for biopolymer conformation. Calculations are under way to work out the contribution to the energies of molecules of interest in biology, associated with both the dihedral angles of the type  $\xi$  and of the type  $\xi'$ .

#### ACKNOWLEDGEMENTS

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us (R. N.) is grateful to the National Council of Educational Research and Training, India, for a scholarship.

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2. IUPAC-IUB, *Commission on Biochemical Nomenclature, Biochemistry*, 1970, 9, 3471.
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### THE ROLE OF 'COSMOLOGICAL CONSTANT' AND $f$ -GRAVITY IN REMOVING GRAVITATIONAL SINGULARITIES

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#### ABSTRACT

In Trautman's recent model of a universe with  $10^{80}$  spin-aligned neutrons, the usual space-time singularity, which is an inherent feature of isotropic general relativistic cosmologies, and the occurrence of which was hitherto considered inevitable in gravitational collapse, is averted by incorporating torsion effects in the usual Einstein field equations through the Einstein-Cartan gravitational theory. Also in another recent paper Salam *et al.* have investigated the effect of taking  $f$ -gravity into account in Trautman's model. However, the severe difficulty of finding a suitable mechanism for aligning perfectly all the nuclear spins of the  $10^{80}$  collapsing hadrons still persists. We suggest an alternative way which does not suffer from this drawback by invoking a "cosmological" constant  $\Lambda$  suitably defined to incorporate short-range  $f$ -gravity. It is shown that the singularity is then avoided and the results obtained by the above authors can be reproduced.

It is generally accepted, especially after the work of Penrose and Hawking<sup>1</sup>, that gravitational collapse inescapably leads to space-time singularities. The singularity theorems imply the inevitability of unlimited collapse to a singular state. It was also known from the much earlier work of Oppenheimer and Snyder<sup>2</sup> that the spherically symmetric gravitational collapse of a dust cloud gives rise to a similar singularity. The occurrence of such singularities is an inherent feature of Einstein's general relativity. Again, the usual isotropic evolutionary models of the universe (Robertson-Walker models) are singular, *i.e.*, at some instant of time they pass through a singular state (interpreted sometimes as the 'initial' state in big-bang models) when matter is collapsed to a physically meaningless infinite density. Modified versions of general relativity such as the Brans-Dicke theory also predict such singularities.

However, Trautman<sup>3</sup> has recently indicated that such singularities may be avoided by directly including the influence of spin on the space-time geometry, *i.e.*, by incorporating the effects of

Cartan's torsion in the Einstein-Cartan theory of gravitation, which is a generalization of Einstein's general relativity. This theory which essentially involves the addition of torsion terms to the usual Einstein equations was originated by Cartan<sup>4</sup> and independently worked out by Sciama<sup>5</sup> and Kibble<sup>6</sup>. Here the geometry of space-time is determined by a metric tensor and linear connection fields which are independently varied in the usual Palatini form of the action integral. In the absence of sources the connection reduces to the ordinary Christoffel symbol but with sources included the resulting equations imply an additional torsion term apart from the Riemannian connection. Unlike Einstein's theory the torsion tensor  $\Omega_{\rho}{}^{\mu}$  is not required to vanish but is related to the density  $S_{\nu\rho}{}^{\mu}$  of an intrinsic angular momentum source. The field equations then become<sup>6,7</sup>:

$$R_{\mu\nu} - \frac{1}{2} g_{\mu\nu} g^{\rho\sigma} R_{\rho\sigma} = 8\pi G c^{-4} T_{\mu\nu}, \quad (1)$$

$$\Omega_{\nu\rho}{}^{\mu} + \delta_{\nu\rho} \Omega_{\sigma}{}^{\sigma} - \delta_{\rho}{}^{\mu} \Omega_{\sigma\nu}{}^{\sigma} = 8\pi G c^{-4} S_{\nu\rho}{}^{\mu}. \quad (2)$$

Here  $R_{\mu\nu\rho\sigma}$  is the curvature tensor formed from the connection,  $R_{\mu\nu} = g^{\rho\sigma} R_{\rho\mu\nu\sigma}$ ,  $g_{\mu\nu}$  is the metric



tensor and  $T_{\mu\nu}$  is the asymmetric energy momentum tensor. If the intrinsic angular momentum density  $S_{\nu\rho}{}^\mu$  is neglected, Eqs. (1) and (2) reduce to the usual Einstein field equations.

Earlier work of Kopczynski<sup>7</sup> showed that the usual Friedmann singularity in cosmological models is avoided with a solution of the Einstein-Cartan equations with a spherically-symmetric distribution of spins. Kopczynski also constructed a class of non-singular cosmological models based on the Einstein-Cartan theory which provide a lower bound for the minimum radius of the universe. In Trautman's model, the universe is filled with spinning dust characterized by its four-velocity  $u^\mu$ , mass density  $\rho$  and spin density  $S_{\nu\rho}{}^\mu = u^\mu \bar{S}_{\nu\rho}$ .  $u^\rho \bar{S}_{\nu\rho} = 0$ , these assumptions being compatible with the isotropy of the Robertson-Walker line element. Now assuming that the spins of all the  $10^{80}$  neutrons in the universe are aligned along the  $x$ -axis, Trautman reduces Eq. (1) to the modified Friedmann equation :

$$\frac{1}{2} \dot{R}^2 - \frac{GM}{R} + \frac{3G^2 S^2}{2c^4 R^4} = 0, \quad (3)$$

where  $M$  is the total conserved mass,  $\frac{4}{3}\pi \rho R^3$ , and  $S$  is the total spin (as all spins are assumed to be perfectly aligned) given by  $S = \frac{4}{3}\pi\sigma R^3$ ,  $\sigma$  being a spin density. The last term in Eq. (3) modifies the usual Friedmann relation and is equivalent to a "repulsive potential" dominant at small values of  $R$ . This term prevents the solution from approaching zero. Thus Trautman is able to show that at  $t = 0$ , the minimum radius of a sphere containing  $N$  particles of mass  $m = M/N$  and spin  $\frac{1}{2}\hbar = S/N$  is :

$$R_{\min} = (3NG\hbar^2/8m_N c^4)^{1/3}. \quad (4)$$

$m_N$  being the neutron mass. For  $N = 10^{80}$ , believed to represent the total number of baryons in the accessible universe,  $R_{\min}$  is of the order of 1 cm ! The corresponding density of matter at  $t = 0$ , is of the order of  $m_N^2 c^4 / G \hbar^2 \simeq 10^{55}$  g.cm.<sup>-3</sup>. Trautman points out that this low value for  $R_{\min}$  is much greater than the Planck length  $(\hbar G/c^3)^{1/2} \simeq 10^{-33}$  cm., at which stage quantum fluctuations of the gravitational field become significant and quantum gravitational effects are supposed to play an important role<sup>8-10</sup>. Also the density at  $t = 0$  in Trautman's model is much smaller than the absurdly high density  $c^5/G^2\hbar \simeq 10^{94}$  g.cm.<sup>-3</sup> at which quantum effects of the gravitational field dominate. Thus, inclusion of quantum gravitational effects in classical general relativistic collapse and cosmology avoids the infinite density of the singularity but gives it the absurdly high density of  $10^{94}$  g.cm.<sup>-3</sup>. But by including torsion in classi-

cal general relativity, Trautman has brought down the cosmological density at  $t = 0$  still further to  $10^{55}$  g.cm.<sup>-3</sup> corresponding to the size of 1 cm. But the serious difficulty with Trautman's model is the need for a suitable mechanism to effect the perfect spin alignment necessary to get his results. A possible mechanism suggested by him is the presence of a cosmic (intergalactic) magnetic field—which may successfully compete with the increasing temperature  $T$  as the collapse proceeds, provided the flux is conserved and  $HR^2 = \text{constant}$ . As  $RT = \text{constant}$ , (i.e., black-body radiation),  $\mu H/kT$  behaves like  $1/R$  and might have been initially large enough to align all spins. However, the presence of such an intergalactic magnetic field is highly doubtful and there are difficulties associated with the exclusion principle in case such a perfect alignment at such high temperatures (near  $t = 0$ ) is ever possible. Moreover, the intense magnetic field necessary for the model will also significantly contribute to the energy-momentum tensor. This has not been taken into account by Trautman. As the collapse proceeds increasing alignment of spins is necessary to provide sufficient torsion to brake the collapse.

In a recent work, Narlikar<sup>11</sup> has suggested that the problem of singularities should be resolved by a proper consideration of matter creation through a negative energy C-field. By incorporating the C-field energy tensor in the usual Einstein field equations he obtains a non-singular Friedmann model with the same  $R(t)$  as obtained by Trautman.

This can be understood as follows: In general relativity the presence of the negative energy C-field amounts to repulsion, and this causes one of the main requirements of Penrose and Hawking to break down thus avoiding the singularity. However, the evidence for a C-field and the actual occurrence of creation of matter in the universe is highly controversial.

In a recent paper, Isham, Salam and Strathdee<sup>12</sup> have investigated the effect on Trautman's results when  $f$ -gravity is taken into account, i.e., Cartan's formulation is taken into account for both  $f$  and  $g$  fields. They have derived the field equations used by Trautman from Lagrangian field theory, i.e., from a variational principle. By considering the interaction of a Dirac spinor with the vierbein gravitational field and spinor connection it is indicated that the torsional effect in Trautman's model manifests itself by the appearance in the Lagrangian of an effective spin-spin contact interaction term  $\kappa^2 (\bar{\psi}\gamma_\mu \gamma_5 \psi)^2 (-g)^{1/2}$  proportional to the Newtonian gravitational constant. Previously, the appearance of such a non-minimal term in the Palatini-type Lagrangian when spinor fields are

coupled to gravity was emphasized by various authors such as Weyl<sup>13</sup>, Sciama<sup>5</sup> and Kibble<sup>6</sup>. This corresponds to Trautman's term which arrests collapse to a singularity as discussed before. Earlier Salam *et al.* had suggested<sup>14</sup> that the natural vehicle for taking hadronic short-range forces into consideration in gravitational physics was through a two-tensor ( $f$ - $g$ ) theory of gravity, i.e., in addition to Einstein's massless graviton field  $g_{\mu\nu}$  (mediated by massless gravitons) we have to consider the massive strong gravity field  $f_{\mu\nu}$  mediated by massive spin  $2^+$   $f$ -mesons. The gravitational interaction between hadrons can proceed *via*  $f$ -mesons with  $f$ -gravity coupling to hadronic matter. They have shown that the interposition of  $f$ -gravity on Trautman's work has a profound effect and their chief result is that the spin-aligned hadronic matter will now collapse to a minimum radius of  $\simeq 10^{13}$  cm, rather than 1 cm which is the minimum radius of the Trautman universe. This implies that the maximum matter density in Trautman's universe of  $10^{80}$  neutrons with their spins aligned is  $\simeq 10^{17}$  gm cm<sup>-3</sup> rather than  $10^{55}$  gm cm<sup>-3</sup>. Thus hadronic matter can collapse to densities only one or two orders of magnitude higher than nuclear densities. As most astrophysical and cosmological models of superdense matter<sup>15</sup> involve a hadron gas composed predominantly of hadrons, it seems reasonable to invoke  $f$ -gravity with its coupling constant  $G_f$  rather than the Newtonian constant  $G$ . However the difficult problem of perfect alignment of all hadron spins still remains a severe drawback as in the case of the Trautman model. We now suggest a way out of the difficulty. We shall obtain both the results of Trautman and Salam *et al.*, without this requirement.

In an earlier work<sup>16</sup> we had reinterpreted the 'cosmological constant'  $\Lambda$  occurring in Einstein's equations in terms of the inverse Compton length of the  $f$ -meson. This seemed to be the most natural way to incorporate the short-range  $f$ -gravity *via*  $f$ -mesons into Einstein's field equations.

Thus

$$\Lambda \simeq \left( \frac{m_f c}{\hbar} \right)^2. \quad (5)$$

Then for a spherically-symmetric collapse we can write for the potential: ( $\Lambda$  is positive)

$$V = c^2 (g_{00} - 1) = c^2 \left( -\frac{2GM}{Rc^2} - \frac{\Lambda R^2}{3} \right).$$

To find the equilibrium value of the radius ( $R_{eq}$ ) we require the potential gradient to vanish and thus all forces to vanish. This gives

$$\ddot{R} = 0 = \frac{2GM}{R^2 c^2} - \frac{\Lambda R}{3} = 0 \quad (6)$$

or

$$R_{eq} = \left( \frac{3GM}{\Lambda c^2} \right)^{1/3} = \left( \frac{3GM^2 \hbar^2}{m_f^2 c^4} \right)^{1/3}. \quad (7)$$

More precisely, the usual Friedmann equation with a cosmological term  $\Lambda$ , (for an Einstein-DeSitter universe with zero pressure and Euclidean 3-space) is:

$$\dot{R}^2 = \frac{8\pi G \rho}{3} R^2 - \frac{\Lambda c^2}{3} R^2. \quad (8)$$

Putting  $M = (4/3)\pi R^3 \rho$ ,  $R = 0$ , at  $t = 0$ , gives:

$$R(0) = \left( \frac{3GM}{\Lambda c^2} \right)^{1/3},$$

the same as in Eq. (7), where  $M$  is the total mass of the  $10^{80}$  baryons in the universe (each baryon having a mass  $\simeq 10^{-24}$  g) and  $\Lambda$  is as defined in Eq. (5), where  $m_f \simeq 1500$  MeV. Substitution of these values in Eq. (7) yields:  $R(0) \simeq 1$  cm (!) and the corresponding density of matter is given by  $\rho = \Lambda c^2 / 4G \simeq 10^{55}$  g cm<sup>-3</sup>. These values are the same as those in Trautman's model. The  $\Lambda$ -term appearing in Eqs. (6) and (8) is of the same order of magnitude as the torsion term in Trautman's model. In both cases these additional terms (torsion in Trautman's, the redefined  $\Lambda$  in ours) in the Friedmann equation are the ones which avert the singularity. For  $\Lambda = 0$ , i.e., for the massless gravity field, of infinite range, we recover the old singularity,  $R = 0$ , from Eq. (8).

If we invoke  $f$ -gravity, the Newtonian constant  $G$  must necessarily be replaced by the corresponding coupling constant  $G_f$  for  $f$ -gravity, which was estimated in an earlier work<sup>17</sup> to be of the order of  $10^{39}G$ . Therefore replacing  $G$  in Eq. (7) by  $G_f$  we get  $R(0) \simeq 10^{13}$  cm and the corresponding matter density turns out to be  $10^{17}$  g cm<sup>-3</sup>; these results being the same as those obtained by Salam *et al.*<sup>12</sup>!

We note that in our formulation the difficult hurdle of finding a suitable mechanism for the alignment of all hadron spins is not encountered.

We can try to understand these results as follows: The Hawking-Penrose<sup>1</sup> singularity theorems for the Einstein field equations  $G_{\mu\nu} + \Lambda g_{\mu\nu} = K T_{\mu\nu}$  which can be derived from the Lagrangian

$$\mathcal{L} = (-g)^{1/2} \kappa (R - 2\Lambda) + \mathcal{L}_{\text{matter}}, \quad (9)$$

do not necessarily hold if the cosmological constant is positive. Indeed it is remarked in Ref. (13) that for all spins aligned in the same direction, the effective spin-spin interaction term in the Lagrangian for the Einstein-Cartan theory which forms the essence of Trautman's torsion term can be regarded as being of the form  $\bar{L} = -(-g)^{1/2} A_{eff}(x)$ , where

$\Lambda_{eff}(x)$  is a positive effective "cosmological" contribution to the Lagrangian. As we remarked earlier the  $\Lambda$  term in Eqs. (6) and (8) is of the same order of magnitude as Trautman's torsion term.

Also it is interesting to note that by using  $G$ , instead of  $G$ , the absurdly high density  $c^3/G^2\hbar \simeq 10^{94}$  g. cm.<sup>-3</sup>, at which quantum effects of the gravitational field and of space-time curvature are expected to become important is reduced (scaled down) to  $\simeq 10^{17}$  g. cm.<sup>-3</sup>, the same value as quoted above. This enables us to explain the somewhat paradoxical result of our getting a much larger radius  $R(0)$  and a much lower density  $\rho(0)$  on using the higher coupling constant  $G_1$  when one would have expected the reverse to happen, i.e., lower radius and higher densities due to the larger gravitation constant, (and hence stronger collapse). The answer is that for  $f$ -gravity the quantum effects which avert the singularity and halt the collapse (essentially through the  $\Lambda$  term) become important at much lower densities ( $\simeq 10^{17}$  g. cm.<sup>-3</sup>) and hence larger radii of the collapsing matter than in classical GTR when these effects become important only at densities  $\simeq 10^{94}$  g. cm.<sup>-3</sup> corresponding to much lower initial radii. For classical GTR with  $\Lambda = 0$  and without consideration of quantum effects, the density of the singularity is infinity corresponding to  $R(0) = 0$ . That the  $\Lambda$  term can avert total collapse is indicative of a repulsive interaction effect. In fact, in a recent work it has been shown that the theory with  $\Lambda$  is related to massive scalar mesons and spin 2+ tensor mesons, with the former being more massive. Also, the former seems to have a repulsive shorter-range

interaction<sup>18</sup>. A brief account of the work reported here appears in Nature<sup>19</sup>.

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## KAEMMERERITES FROM CHROMITE DEPOSITS OF BYRAPUR, HASSAN DISTRICT, KARNATAKA STATE

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### ABSTRACT

Two physically distinct varieties of chromium chlorites occur in the chromite deposits of Byrapur, which form a part of the Naggehalli schist belt. They differ in their habit, one being flaky and the other lamellar to fibrous. Cr<sub>2</sub>O<sub>3</sub> in the flaky chlorite is 3.9% and in the lamellar it is 4%. Based on the spacing  $d_{007}$  and intensity  $d_{002}$  reflections (Lapham, 1958), the Cr is believed to be in the octahedral coordination in both the varieties and hence identified as kaemmererites.

**THE** Naggehalli schist belt of Karnataka State extending over a length of 40 km. (Lat. 12° 58' 30"–13° 17' 43" and Long. 76° 17' 20"–76° 29' 30") and a width of about 1.5 km. is composed of green schists, amphibolites, serpentinites, and lenses of

dunite transected by siliceous and carbonate veins. The ultramafic lenses contain rich chromite deposits. In association with these chromite deposits, the occurrence of chromium chlorites has been reported earlier from Hulikere chromite mines near Jambur,

Hassan District (Viswanathiah<sup>7</sup>, 1951; Varadarajan<sup>6</sup>, 1964) and chromite mines of Byrapur, Hassan District (Varadarajan<sup>5</sup>, 1957).

The authors during the course of their field work encountered chromium chlorites that were distinct in their habit, flaky and lamellar, associated with the ultramafites of Byrapur chromite mines, Hassan District. The flaky form occurs as lensoidal lumps in carbonate veins or as thin films bordering these veins which transect the chromite ore. The lamellar form is always associated with the carbonate and serpentine veins, where the lamellae are aligned across the direction of the veins. These forms were earlier identified by Vardarajan<sup>5</sup>, (1957) as kaemmererite (flaky) and kotschubeite (lamellar), based on their tetrahedral Al content and Fe/Fe + Mg ratio.

X-ray powder data, chemical and differential thermal analysis for the two forms, and their identification based on their crystallochemical characteristics are presented in this paper.

**Physical and Optical characteristics.**—Chlorites of both flaky and lamellar habits are light to deep purple in colour. The flaky variety is more elastic and could be cleaved into thin flakes, whereas the other variety is lamellar to fibrous and rather brittle. Both are non-pleochroic, show anomalous deep blue to violet interference colours and may appear almost isotropic. They are essentially uniaxial negative, though a few grains evidence biaxial isogyres. The optic axial angle for such grains is about 5°. The intimate association and reaction of carbonate material with the chlorites is clear in thin-section.

**Instrumental Technique.**—Both the samples were carefully purified using bromoform suitably diluted with benzene in order to remove the associated carbonate and other impurities. The sample was further checked and hand picked under binocular microscope. An X-ray powder diffractogram was taken using Shimadzu XRD Unit with CuK $\alpha$  radiation. The 2 $\theta$  scanning rate was 1° per minute. Quartz was mixed as internal standard. Chemical analysis was carried out using spectrophotometric method of Riley<sup>4</sup> (1958). Chromium was determined volumetrically by the method of Mall<sup>3</sup> (1964). The D.T.A. was carried out on non-recording type of unit, manually programmed with an automatic transformer to give a heating rate of 10°–12° C per minute. The differential temperature was read on the deflecting galvanometer and the temperature measured with vernier potentiometer. 0.5 gm of powder ground with agate pestle and mortar for about an hour was taken in a stainless steel sample holder. Sintered alumina powder was used as reference material.

**Classification.**—The chemical analysis and structural formulae are calculated for these two chlorites on the basis of 18 oxygen atoms Table I. CaO and TiO<sub>2</sub>

TABLE I

*Chemical analysis and structural formulae (Hydrous basis 18 Oxygen Atoms) for Flaky (A) Lamellar (B) Kaemmererites of Byrapur*

Oxides		A	B
SiO <sub>2</sub>	..	32.47	31.66
Al <sub>2</sub> O <sub>3</sub>	..	10.57	10.94
Cr <sub>2</sub> O <sub>3</sub>	..	3.90	4.00
Fe <sub>2</sub> O <sub>3</sub>	..	0.18	0.37
FeO	..	0.84	0.84
MnO	..	0.21	0.26
MgO	..	37.91	38.16
CaO	..	1.41	1.28
TiO <sub>2</sub>	..	0.26	0.33
H <sub>2</sub> O <sup>+</sup>	..	10.72	11.38
H <sub>2</sub> O <sup>-</sup>	..	0.78	0.48
TOTAL	..	99.25	99.70

(A) (Si<sub>3.023</sub> Al<sub>0.977</sub>) (Mg<sub>5.26</sub> Al<sub>0.184</sub> Fe<sup>3+</sup><sub>0.012</sub> Fe<sup>2+</sup><sub>0.065</sub> Cr<sup>3+</sup><sub>0.286</sub> Mn<sub>0.016</sub> O<sub>8</sub> (OH)<sub>6.662</sub>).

(Si Al)<sub>4</sub> (Mg Al Fe<sup>3+</sup> Fe<sup>2+</sup> Cr Mn)<sub>6.823</sub> O<sub>8</sub> (OH)<sub>6.662</sub>

(B) (Si<sub>3.105</sub> Al<sub>0.895</sub>) (Mg<sub>5.576</sub> Al<sub>0.370</sub> Fe<sup>3+</sup><sub>0.027</sub> Fe<sup>2+</sup><sub>0.069</sub> Cr<sub>0.31</sub> Mn<sub>0.021</sub> O<sub>8</sub> (OH)<sub>7.442</sub>).

(Si Al)<sub>4</sub> (Mg Al Fe<sup>3+</sup> Fe<sup>2+</sup> Cr Mn)<sub>6.373</sub> O<sub>8</sub> (OH)<sub>7.442</sub>

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are suspected to be due to impurities and omitted in the calculation of structural formulae. The Al was first assigned to tetrahedral site assuming complete tetrahedral occupancy and the remainder Al was assigned to octahedral site. Both the chlorites are relatively rich in Mg and Si. They are poor in Al and Fe content and are similar to the chlorites associated with Alpine chromites and ultramafites (Lapham, personal communication). The Cr<sub>2</sub>O<sub>3</sub> in flaky and lamellar forms is 3.9 and 4% respectively. The presence of CaO in the analysis and relatively higher magnesium content is attributed to the intimately interleaved magnesite and calcite, which could not be completely got rid off. There is an apparent charge imbalance with

the deficiency of hydroxyl molecules, particularly in the flaky chlorite.

Samples	I endo- thermic	II endo- thermic	exothermic
Flaky (A)	710° C	770° C	837° C
Lamellar (B)	702° C	817° C	846° C

Figure 1 gives the D.T.A. plot of the two Byrapur chlorites. The peak temperatures are given below :

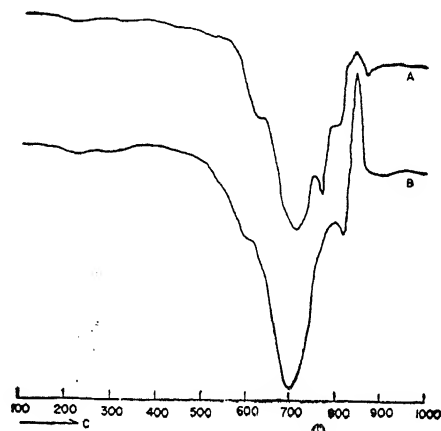


FIG. 1. D.T.A. of flaky (A) and lamellar (B) kaemmererites.

The first two endothermic peaks, corresponding to the expulsion of water from brucite and talc layers respectively, are better resolved in the flaky variety than in the lamellar one. The exothermic decomposition reaction in the lamellar is sharper and more vigorous than in the flaky chlorite. The temperature of decomposition is shown to decrease with increasing chromium content (Lapham<sup>2</sup>, 1958). On the other hand in the Byrapur chlorites with an increase of 0.1% of  $\text{Cr}_2\text{O}_3$  (sample B) the decomposition temperature at about 850° C has increased by 9° and the second endothermic reaction at about 800° C representing destruction of talc layer, by 47° C.

X-ray powder data for the two Byrapur chlorites was obtained with quartz as internal standard, (Table II). The associated carbonate material was identified with powder photographs and is mainly composed of magnesite and a minor amount of calcite.

Lapham<sup>2</sup> (1958) suggested a new classification for chromium chlorites based on the crystallochemical characteristics which appear with the introduction of chromium in the structure. Chromium according to him, could occupy a tetrahedral site replacing silica-alumina, or an octahedral site

TABLE II  
Powder data of flaky (A) and lamellar (B) Kaemmererites of Byrapur along with the spacings of internal standard quartz.

A		B		Quartz XPDF 5-490	
d Å	I/I <sub>0</sub>	d Å	I/I <sub>0</sub>	d Å	I/I <sub>0</sub>
14.255	25	14.325	29	..	..
7.179	100	7.181	100	..	..
4.783	59	4.770	67	..	..
4.260	21	4.265	20	4.26	35
*3.978	16	..	..	..	..
3.590	94	3.583	98	..	..
3.336	45	3.348	38	3.343	100
2.870	23	2.867	24	..	..
2.548	13	2.549	16	..	..
*2.495	12	2.509	15	..	..
2.449	14	2.451	15	2.458	12
2.399	13	2.386	12	..	..
2.281	10	2.281	12	2.282	12
2.239	9	2.239	10	2.237	6
2.127	9	2.105	14	2.128	9
2.050	12	2.049	13	..	..
2.014	11	2.014	14	..	..
..	..	*1.940	10	1.980	6
..	..	1.895	9	..	..
1.818	11	1.820	11	1.817	17
1.800	9	..	..	1.801	< 1
..	..	1.703	11	..	..
1.670	9	1.673	10	1.672	7
..	..	1.629	9	1.659	3
..	..	1.578	12	1.608	< 1
1.541	13	1.542	15	1.541	15
..	..	1.507	9	..	..
..	..	1.490	8	..	..
1.430	11	1.435	11	1.453	1
..	..	1.406	12	1.418	1
1.382	9	1.383	9	1.382	7
1.375	9	1.375	10	1.375	11
..	..	..	..	1.372	9
..	..	1.299	8	1.288	3
..	..	1.230	8	1.256	4
1.199	9	1.196	10	1.199	5
1.181	8	1.182	9	..	..

Plus ten lines to 1.0346

\* Impurities.

replacing magnesium-iron, this distinction being measurable only when the chromium exceeds 2%. Accordingly chlorite with chromium in the tetrahedral site may be classified as kotschubeite and those in the octahedral site as kaemmererites. An earlier definition distinguished these two varieties by Si/Al ratio (Hey<sup>1</sup>, 1954) in which case kotschubeite resembles clinocllore and kaemmerite resembles peninite. Lapham retained these terms for chlorites with chromium less than 2% and

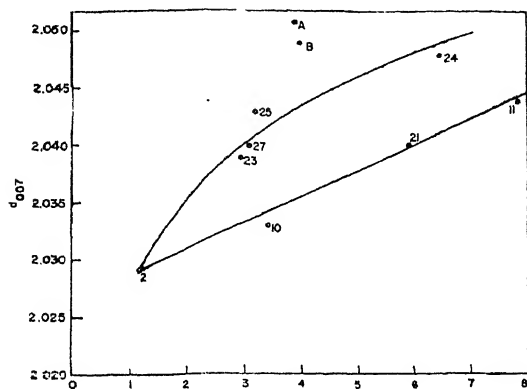


FIG. 2. Variation of chromic oxide content with (007) spacing. The upper curve represents octahedral chromium and the lower curve tetrahedral chromium (after Lapham, 1958).

suggested the use of names peninite and clinocllore by prefixing the term 'chromium'.

The plot of  $\text{Cr}_2\text{O}_3$  against  $d_{007}$  and  $\text{Cr}_2\text{O}_3$  against intensity of 002 reflections for Byrapur chlorites on the diagrams of Lapham (Figs. 2 and 3) indicate that chromium in both the flaky and lamellar chlorites is in the octahedral position. The presence of more than 2% chromium and its occupancy in the octahedral sites warrants the classification of both the flaky and lamellar chlorites of Byrapur as kaemmererites.

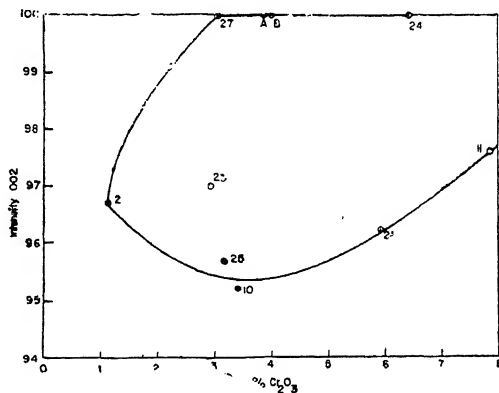


FIG. 3. Variation in chromic oxide content with (002) intensity. The upper curve represents octahedral chromium and the lower curve tetrahedral chromium (after Lapham, 1958).

The authors are thankful to: Dr. Davis M. Lapham, Bureau of Topographic and Geologic Survey, Pennsylvania, for critically reading through the manuscript and for his useful suggestions.

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## DEVELOPMENT OF CULTURED PEARLS IN INDIA

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THE technology of production of spherical cultured pearls was first developed in Japan in 1907<sup>1</sup>. In Australia, the first experimental production of cultured pearls was reported in 1957<sup>2</sup>. In India, experiments to produce cultured pearls commenced in 1933 but, despite prolonged efforts, were not successful<sup>3</sup>. The present author, in an earlier communication, described the pearl culture technology of Japan and indicated the prospects of producing cultured pearls in the Indian pearl oyster *Pinctada fucata*<sup>4</sup>. Experiments on pearl culture were initiated at the Pearl Culture Laboratory of the Central Marine Fisheries Research Institute at Veppalodai (near Tuticorin) in December 1972. The successful development of the

technology, for the first time in India, is described here.

### EXPERIMENTAL PROCEDURE

A pearl oyster farm was established in the Gulf of Mannar, about 1.5 km off Veppalodai. Modern method of raft culture was employed for rearing the oysters which were collected from the pearl banks off Tuticorin. The pearl oysters, after they have grown in the farm for a few months, were brought to the laboratory for surgical operation. A total of 150 oysters was operated in the first series of experiments during May-August 1973. The oysters ranged 53.7–69.0 mm along their dorso-ventral axis and 25.5–51.5 g in weight.

The oysters were kept in a fibreglass tank holding about 1000 litres of sea water for the initial conditioning. On the day of operation they were removed in batches, cleaned and placed in glass troughs containing sea water. Menthol crystals were spread over the surface for further conditioning. When the oysters were found adequately narcotised, they were taken out one by one and clamped on a special stand for the operation. The shell-opening was regulated to an optimum gap with a pair of tongs. A healthy unconditioned oyster was opened and portions of mantles were cut. Small bits of about 2 mm square were prepared from the mantle and kept moist under aseptic conditions to be used as graft tissues. Spherical shell beads used as nuclei were obtained from Japan and these were of 3-4 mm diameter. Larger nuclei of 6 mm diameter made indigenously from the conch shell were also used in a few cases to study their suitability.

pletion of the operation the oysters were returned to sea water in basins.

Within minutes of their immersion in sea water, the oysters began to show signs of recovery. They were kept for a week in the laboratory in wooden vats arranged in a series in which a constant flow of sea water was maintained. There were a few instances of ejection of nuclei in the early stages of the experiments. Subsequently, the oysters were returned to the raft in the farm. During the post-operative phase of culture, the oysters were periodically taken out and cleaned of the fouling organisms.

### RESULTS

The operated oysters were examined in batches for the development of cultured pearls. The results obtained in 77 oysters so far examined are presented in Table I.

TABLE I

*Results of experiments on production of cultured pearls*

Batch No.	Date of operation	Date of examination for pearls	Duration of post-operative culture (days)	No. of oysters examined	No. of oysters		
					Nucleus ejected	Nucleus present, but no pearl	Pearls produced
I	12-6-1973 25-6-1973	25- 7-1973	30-43	11	5	4	2
II	25-6-1973 2- 7-1973 10-8-1973	18-10-1973	69-108	16	3	7	6
III	10-8-1973 14-8-1973	16-11-1973	94-98	12	2	3	7
IV	14-8-1973 16-8-1973	22- 1-1974	159-161	18	3	4	11*
V	16-8-1973	24- 1-1974	161	11	3	-	8
VI	16-8-1973	23- 2-1974	191	9	-	-	9
Total				77	16	18	43

\* Of the 11 pearls, two were produced with conch shell nuclei made indigenously.

During the operation, an incision was made at the base of the foot and a graft piece was carefully inserted within the tissues of the oyster. This was followed by the implantation of a nucleus with the aid of special instruments. The graft piece and the nucleus were brought in contact with each other as far as the two could be manipulated. Gonads and the alimentary canal of the oyster were the two sites used for implanting the nuclei. On the com-

The above cultured pearls, though removed from the oysters sooner than the intended period of culture with a view to assessing the success of the experiments, form the first series of free, spherical cultured pearls produced in India. Deposition of nacre over the nucleus was observed 30 days after the operation and the pearl had a distinct lustre after 43 days. Those produced between 69 and 191 days were of bright lustre. The results

of the experiments show a progressive improvement in the techniques employed in the operation. The



FIG. 1. The Indian pearl oyster *Pinctada fucata* with a cultured pearl *in situ* (indicated by arrow).

ratio of 8 pearls out of 11 oysters obtained in batch V is considered to be extremely good. Cent per cent success was achieved in batch VI. Pearls

were produced both in the regions of gonads and alimentary canal. Pearls of different colours, namely, silver white, ivory white, light pink, golden yellow and steel grey, have been obtained among those so far produced. The growth of pearl has been found to be faster in the warmer waters of the Gulf of Mannar than in the well-known colder areas of Japan. These experiments prove that an industry of cultured pearls can be developed in India entirely from indigenous efforts.

The author is extremely grateful to Dr. S. Z. Qasim for his very keen interest, kind encouragement and inspiring discussions which were responsible for the rapid success of the experiments. His thanks are to Shri T. V. Venkataraman, Director of Fisheries, Madras, for his interest in the work.

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## LETTERS TO THE EDITOR

RADIO-METRIC DATING OF THE  
DALHOUSIE GRANITE

In recent years, radio-metric dating has become an important tool for research in earth sciences. However, not much work has been done in this field in India. In particular, only scanty radio-metric data is available for the vast Himalayan terrain. Whatever is available consists mainly of mineral ages, obtained by use of K-Ar technique through analyses in laboratories outside India. Recent studies<sup>1</sup> in case of many samples belonging to poly-metamorphic regions have indicated an excess or loss of radiogenic Argon resulting from its diffusion from the original source over appreciable distances during the long geological time. As an example, Saxena and Miller<sup>2</sup> obtained apparent ages as large as 4100 m.y. and 4250 m.y. for two minerals of N. W. Himalaya. The authors stated that significance of these apparent ages was doubtful. This is an indication that K-Ar mineral ages for the Himalayan terrain require a very careful scrutiny and interpretation. In comparison, Rb-Sr whole rock method has been demonstrated<sup>1</sup> to be, in general, free from similar problems.

For the Himalaya, the only whole-rock Rb-Sr data so far available is that of Jager *et al.*<sup>3</sup> who determined an age for the Mandi granite of Himachal Pradesh (India). The present communication describes the preliminary results for the age of the Dalhousie granite, H.P., by use of this technique. PHS/17 is one of the first samples which has been analysed. This is a coarse-grained granite which contains a fairly good amount of biotite as well as muscovite. Small-sized porphyroblasts of white feldspars are also present. This sample was collected from Matuna (Longitude 76°; Latitude 32.5°) near Dalhousie.

The data has been obtained by use of a Nier-type of Mass spectrometer of 25 cm radius equipped with an ion-source for solid samples, which was fabricated and provided to us at Chandigarh by Bhabha Atomic Research Centre, Bombay. Pending the availability of an isochron, the initial value for  $\text{Sr}^{87}/\text{Sr}^{86}$  was taken as 0.7091. Decay constant for  $\text{Rb}^{87}$  was taken as  $1.47 \times 10^{-11}$  per year. Our analyses have given the following results:

$\text{Rb}^{87} = 94.5$  ppm;  $\text{Sr}^{87}$ , radiogenic = 0.633 ppm  
Common Sr = 33.6 ppm;  $\text{Sr}^{87}/\text{Sr}^{86} = 0.890 \pm 0.015$

Apparent Age =  $456 \pm 50$  million years.

The above value agrees within experimental errors with the age of the Mandi granite, viz.,  $500 \pm 100$  m.y., obtained by Jager *et al.*<sup>3</sup>. It is quite likely that the two outcrops are of equivalent age.

Work is in progress for obtaining an isochron-age for the Dalhousie granite. This is estimated to be a few percent lower than the value given above. There is no doubt, whatsoever, that the Dalhousie granite is not a tertiary granite.

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DEPENDENCE OF SURFACE TENSION OF  
LIQUID HELIUM I ON THE TEMPERATURE  
AND THE DENSITY

It is shown that for liquid helium I (a liquid with a very low boiling point), the dependence of surface tension on the temperature and the density is governed by the well-known relations, such as, the Eötvös rule<sup>1</sup> and the Sugden relation<sup>2</sup>, which are obeyed by the normal non-associated liquids. Further, the values of the Eötvös constant and the "Parachor" for liquid helium I are calculated.

Eötvös has shown that the surface tension of normal non-associated liquids obeys the relation,

$$S = \frac{K_e T_c}{M^{2/3}} \left[ \rho^{2/3} \left( 1 - \frac{T}{T_c} \right) \right],$$

where  $S$  = Surface tension,  $M$  = gram-molecular weight,  $\rho$  = liquid density,  $K_e$  = Eötvös constant,  $T$  = temperature, and  $T_c$  = critical temperature. This relation is tested for liquid helium I by drawing the graph of  $S$  against  $[\rho^{2/3} (1 - T/T_c)]$ . The values of surface tension from Devaraj and Hollis Hallett<sup>3</sup> and the density data from Kerr<sup>4</sup> are used. It is observed that liquid helium I obeys quite well the Eötvös rule. The Eötvös constant ( $K_e$ ) for liquid helium I is calculated from the slope of the straight-line graph and is found to be 1.06.

Sugden's relation shows that for a normal liquid, the quantity  $MS^{1/4}/(\rho_l - \rho_v)$  is a constant over a wide range of temperature and the constant is known as "Parachor" (P) of the substance. Thus

$$\frac{MS^{1/4}}{(\rho_l - \rho_v)} = P.$$

where  $M$ =gram-molecular weight,  $S$ =surface tension,  $\rho_l$ =liquid density, and  $\rho_v$ =density of saturated vapour. Using the surface tension data of Devaraj and Hollis Hallett<sup>2</sup>, the values of liquid density from Kerr<sup>4</sup> and the density data of saturated vapour from Erickson and Roberts<sup>5</sup>, a plot of  $S^{1/4}$  against  $(\rho_l - \rho_v)$  for liquid helium I is made and the Sugden relation is verified. It is observed that all the points lie very well on the drawn straight-line, which on extrapolation passes through the origin. This straight-line can be represented by the equation  $S^{1/4} = 5.20 (\rho_l - \rho_v)$  within the graphical errors. From the slope of this line and the known gram-molecular weight, the "Parachor" of liquid helium I is calculated as 20.8 c.g.s. unit.

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### KINETICS OF CONDENSATION OF 3:5: DIMETHYL 4-NITRO ISOXAZOLE WITH SUBSTITUTED BENZALDEHYDES

The reaction between aromatic aldehydes and ketones to yield chalkones is catalysed both by acids and bases<sup>1-4</sup>. Similarly, the condensations of 3:5: dimethyl 4-nitro isoxazole with various substituted benzaldehydes have been reported to be base catalysed<sup>5</sup>. It has also been shown from a spectroscopic study that the 5-methyl group is reactive and the end product is a 5-styryl derivative of isoxazole<sup>6</sup>. In the present work, some of the kinetic data obtained for base catalysed condensation of 3:5: dimethyl 4-nitro isoxazole with various substituted benzaldehydes under different conditions are presented and discussed. Calculation of rate constants, effect of substituents in the aldehyde moiety and evaluation of thermodynamic parameters comprise new features in our studies not reported earlier.

The 3:5: dimethyl 4-nitro isoxazole was prepared by the method reported earlier<sup>7</sup>. All other

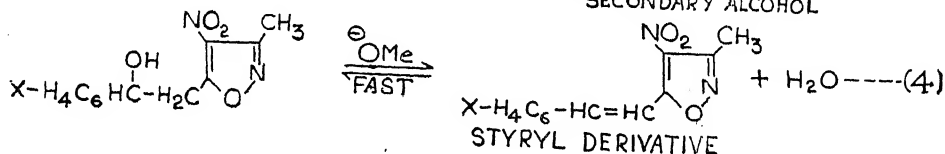
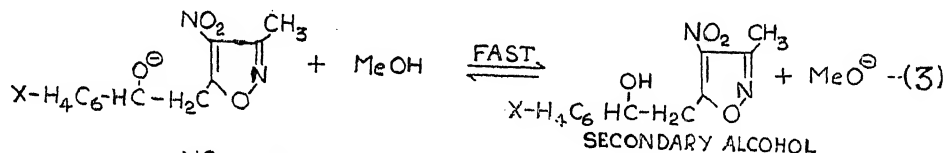
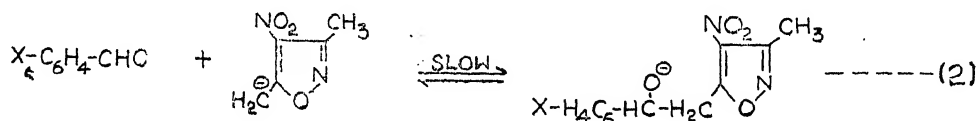
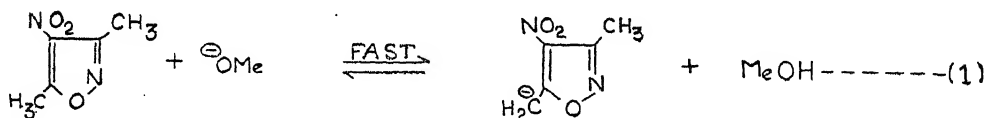
chemicals were of the highest purity and wherever necessary further purification was done by standard methods. Solutions of isoxazole, aldehyde and sodium methoxide of appropriate concentrations in methanol were kept in thermostat for one half hour and mixed just before starting the reaction. Samples were withdrawn periodically and the concentration of styryl derivative determined colorimetrically using Bausch and Lomb 'SPECTRONIC 20' Colorimeter at  $\lambda = 380$  nm, as at this wavelength the styryl derivative obeys Beer's Law. The order with respect to isoxazole, aldehyde and sodium methoxide was determined by the isolation method and found to be one each in all the cases studied. The kinetic data obtained for the base catalysed reaction between 3:5: dimethyl 4-nitro isoxazole and aldehydes is similar to that obtained for the reaction between acetone or ethyl methyl ketone with substituted benzaldehydes<sup>8</sup>. The rate of formation of styryl derivative is affected by substituents in the aldehydes moiety as shown in Table I. Since

TABLE I  
Thermodynamic parameters  
Condensation of substituted benzaldehydes with  
3:5: dimethyl 4-nitro isoxazole with sodium  
methoxide as a catalyst  
Temp. 35°

Substi- tuent in Benzal- dehyde Ring.	$k'' \times 10^3$ lit.mol <sup>-1</sup> sec. <sup>-1</sup>	$\Delta E$ K.cals. mol. <sup>-1</sup>	$\Delta H^\ddagger$ K.cals. mol. <sup>-1</sup>	$\Delta G^\ddagger$ K.cals. mol. <sup>-1</sup>	$\Delta S^\ddagger$ e.u
NIL	6.86	28.83	27.60	21.10	21.1
<i>o</i> -OH	9.50	18.45	17.23	20.90	-12.0
<i>m</i> -OH	15.40	21.97	20.75	20.60	0.4
<i>p</i> -OH	4.19	27.47	26.25	21.27	16.0
<i>p</i> -Cl	17.95	22.89	21.66	20.51	3.7
<i>m</i> -NO <sub>2</sub>	39.60	16.47	15.25	20.02	-19.2
<i>p</i> -NO <sub>2</sub>	50.90	17.40	16.17	19.87	-12.1

the rate is also different from that obtained when acetone or acetophenone is used instead of isoxazole, under otherwise similar conditions, the reaction of aldehyde or isoxazole with sodium methoxide cannot be the rate determining step. In view of the isolation (in some cases) of an unstable intermediate ketol (a secondary alcohol) in the condensation of substituted benzaldehydes with acetone<sup>8-9</sup> or ethyl methyl ketone<sup>10</sup>, it is quite likely that in this case also the formation of a styryl derivative might occur via an intermediate secondary alcohol.

## CHART 1



The mechanism could be as shown in Chart 1.

The rate determining step could be either the condensation step to yield the secondary alcohol or the dehydration of the latter. From the effect of solvent on the condensation of aromatic aldehydes with various substituted acetophenones it was found that upto about 60% v/v of methyl, ethyl or isopropyl alcohol-water mixtures, the dehydration is important and above that it is the condensation step that is the rate determining step<sup>11</sup>. Since there is no possibility of studying the effect of solvent in this reaction because of the low solubility of isoxazole in aquo-organic mixtures and could be studied only in pure MeOH medium, we assume that the condensation step is the rate determining one. This is also in agreement with the results obtained by Noyce and Reed<sup>8</sup>, who found the condensation step in the reaction between acetone and benzaldehyde at 0.1 M sodium hydroxide and 90% v/v alcohol-water mixture to be about ten times slower than the corresponding dehydration step.

Hammett's plot of  $\log k/k^0$  vs  $\sigma$  was also found to be linear with a correlation coefficient of 1.0 and  $\rho$  value 0.8. The  $\Delta E$  values for all the reactions were determined from the slope of the Arrhenius plot in the temperature range 30–50° C.

The thermodynamic parameters for all aldehydes studied (Table I), may be explained on the basis of isokinetic relationship<sup>12</sup>, that for a series of compounds of slightly different structure but undergoing a reaction essentially by the same mechanism, the  $\Delta G^\ddagger$  may be more or less constant with relative

changes in  $\Delta H^\ddagger$  and  $\Delta S^\ddagger$ . The plot of  $\Delta H^\ddagger$  Vs  $\Delta S^\ddagger$  was also found to be linear in accordance with the isokinetic equation  $\Delta H = \Delta H_0 + \beta \Delta S$ , where the slope is equal to the isokinetic temperature ( $\beta$ ) and was found to be 350° K.

The authors wish to thank Prof. N. V. Subba Rao, for helpful discussions and constant encouragement.

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# STUDIES ON METAL COMPLEXES OF 5-METHYL-2-AMINO BENZOTHAIAZOLE

FOUR membered chelate rings have been reported with alkylxanthates<sup>1,2</sup>, dialkylthiocarbamates<sup>3</sup> and 2-mercaptobenzothiazoles<sup>4,5</sup>. Recently, the metal complexes of thiazoles<sup>6,7</sup> and benzothiazoles<sup>8,9</sup> have been studied. Considering these facts, the authors synthesised the complexes of Cu(II), Hg(II) and Co(II) with 5-methyl-2-aminobenzothiazole and their structures were established with the help of infrared spectra and elemental analysis.

In the present communication, 5-methyl-2-aminobenzothiazole (L) was prepared by the method of Hegershoff<sup>10</sup>. The Cupric, Mercuric and Cobalt(II) Chlorides were boiled with the ligand (L) in acetic acid and dilute hydrochloric acid (50%) medium separately. The complexes were precipitated with a saturated solution of sodium acetate and purified.

## Experimental

Infrared spectra of these complexes were recorded on Perkin-Elmer Model-257. Coleman-Analyser was used for the estimation of carbon and hydrogen.

M(C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>S)Cl<sub>2</sub> were obtained with the Cupric, Mercuric and Cobalt(II) chlorides. Their colours, analytical and i.r. data are recorded in the table.

## Discussion

As elemental analysis suggests the metal-ligand ratio in the complexes is 1:1. The i.r. spectrum of the ligand shows two —NH stretching bands at 3420 *b* and 3300 *b* cm<sup>-1</sup>, the ν C=N— stretching peak at 1640 *s* cm<sup>-1</sup> and δ NH frequency at 1610 *m* cm<sup>-1</sup>. The complexes of the type M(C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>S)(CH<sub>3</sub>COO)<sub>2</sub> show the shift of —NH peaks to 3300 and 3200 cm<sup>-1</sup>. The complexes also show the shift of ν C=N— to 1645 cm<sup>-1</sup> δ NH to 1585 cm<sup>-1</sup> together with a additional peak of carboxylate ion at 1615 cm<sup>-1</sup>. These changes in the frequencies of —NH<sub>2</sub> group and a additional peak of carboxylate ion confirm that the donation of two lone pair of electrons through nitrogen atoms and the M  $\begin{matrix} \nearrow \text{OCOCH}_3 \\ \searrow \text{OCOCH}_3 \end{matrix}$  bonds to form the chelates.

The complexes (MLCl<sub>2</sub>) show the shift of ν C=N, ν NH and δ NH frequencies and also the absence of carboxylate ion as recorded in Table I.

TABLE I

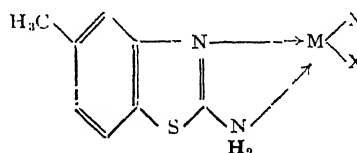
Sl. No.	Complex	Colour	% C		% H		I. R. Assignments			Carboxylate ion
			Found	Calcd.	Found	Calcd.	νNH	δNH	νC=N	
1.	CuLAc <sub>2</sub>	Green	40.60	40.65	4.13	3.95	3,300 <i>b</i> , 3,200 <i>b</i>	1585 <i>w</i>	1645 <i>s</i>	1615 <i>s</i>
2.	HgLAc <sub>2</sub>	White	29.18	29.34	3.01	2.85	3,310 <i>b</i> , 3,205 <i>b</i>	1580 <i>w</i>	1650 <i>s</i>	1600 <i>s</i>
3.	CoLAc <sub>2</sub>	Violet	41.16	41.29	4.12	4.01	3,300 <i>b</i> , 3,190 <i>b</i>	1590 <i>w</i>	1665 <i>s</i>	1620 <i>s</i>
4.	CuLCL <sub>2</sub>	Green	32.25	32.07	2.81	2.67	3,315 <i>b</i> , 3,208 <i>b</i>	1575 <i>s</i>	1650 <i>s</i>	..
5.	HgLCL <sub>2</sub>	White	22.09	22.03	1.96	1.83	3,310 <i>b</i> , 3,208 <i>b</i>	1590 <i>sh</i>	1655 <i>s</i>	..
6.	CoLCL <sub>2</sub>	Violet	32.47	32.60	2.70	2.72	3,320 <i>b</i> , 3,220 <i>b</i>	1570 <i>s</i>	1658 <i>s</i>	..

Where Ac—Acetate, *s*—sharp, *m*—medium, *w*—weak, *sh*—shoulder.

**Preparation of Cu(II) complex of 5-methyl-2-aminobenzothiazole.**—A mixture of the ligand (50 ml, 0.1 M) in acetic acid and cupric chloride (28 ml, 0.1 M) in acetic acid was refluxed for half an hour. A green precipitate was obtained after adding a saturated solution of sodium acetate to the reaction mixture. It was washed with hot water and a little of ethanol. Finally, the complex was washed with Chloroform and dried over CaCl<sub>2</sub>.

Similarly, other complexes were synthesised by using the Mercuric nitrate and Cobalt(II) chloride. However, if dilute hydrochloric acid was used the medium of reaction, the complexes of the type

From the infrared spectral data and analytical results, the most appropriate structure of the complexes would be as shown below :



where X = chloride or acetate.

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#### A PRELIMINARY FORAMINIFERAL REPORT OF THE LOWER TERTIARY ROCKS OF VINJHAN-MIANI AREA, SOUTHWESTERN KUTCH, GUJARAT

PRESENT note gives a brief information of the foraminifera occurring in the Lower Tertiary (Middle Eocene) rocks exposed in the Kankawati river section in the Vinjhan-Miani area (23° 7'; 69° 6', Miani: 23° 6'; 69° 1', Vinjhan).

Srivastava (1970) has given the succession of Lower Tertiary rocks in this area in the following manner :

- |                                                     |                      |
|-----------------------------------------------------|----------------------|
| (6) Fossiliferous light yellow marl (3 m.)          | } Kirthar (Lutetian) |
| (5) Fossiliferous yellow limestone (4 m.)           |                      |
| (4) Unfossiliferous khakhi shales (8 m.)            |                      |
| (3) Conglomerate, bauxite and laterite (3 m.)       |                      |
| (2) Unfossiliferous purplish-brown sandstone (9 m.) | } Laki (Ypresian)    |
| (1) Unfossiliferous cream coloured shales (3 m.)    |                      |

————— Deccan Traps —————

The rock-units<sup>1-4</sup> have not yielded any micro-fauna. The rock-unit<sup>5</sup> is fossiliferous and contains various species of larger foraminifera which are represented by *Nummulites*, *Assilina*, and *Discocyclina* and *Dictyoconoides*. The detailed study of other representatives of this rock-unit is in progress. The rock-unit<sup>6</sup> has yielded a rich assemblage of

planktonic and benthonic foraminifera, some of which have been identified by us and are being enumerated as follows : Planktonic : *Truncorotaloides rohri* Brönnimann and Bermúdez, *Truncorotaloides topilensis* Cushman, *Globorotalia brüdermanni* Cushman, *Turborotalia centralis* (Cushman) and (Bermúdez), *Globigerina yeguaensis* Weinzierl and Applin, *Globigerinatheka* sp., *Inordinatosphaera indica* Mohan and Soodan and *Globigerinoides* sp. Benthonic : *Dorothia*, *Florilus*, *Buliminella*, *Pseudobolivina*, *Eponides*, *Brizalina*, *Textularia*, *Cibicides*, *Asterotalia*, *Pseudonodosaria*, *Trilaculina* and *Nonionella*.

The planktonics of the rock-unit<sup>6</sup> confirm the Middle Eocene (Lutetian) age for it. They are further significant in that they suggest its tentative correlation with Bolli's Middle Eocene biostratigraphic zone—*Porticulasphaera mexicana* zone (now called *Orbulinoides beckmanni* zone) of Eocene Navet Formation of Trinidad (see Bolli, 1957). Our this view finds support in the works of Bandy (1964), Samanta (1969, 1970) and Mohan and Soodan (1970).

Present work forms a preliminary report of the occurrence of some smaller foraminifera in the Lower Tertiary rocks of the area. A detailed work comprising a detailed description of the above-mentioned forms, together with their palaeoecological and biostratigraphic interpretations will be published elsewhere.

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## STUDIES ON THE STRUCTURE OF SILK COTTON

As a part of our continuing studies concerning structure-function relationship in polysaccharides<sup>1</sup>, a detailed study on the structural aspects of silk cotton has been undertaken. Silk cotton, the seed-hair from the pods of silk cotton tree, *Ceiba pentandra* Gaertn. (Bombacaceae), finds use mostly as a filling material, and in sound and thermal insulation. But on account of its poor tensile strength it has limited use as a fibre in the textile industry<sup>2</sup>.

Silk cotton is reported to be composed of cellulose<sup>2</sup> (61-64%) and hemicellulose<sup>3</sup> (20%), and the rest being lignin and other miscellaneous materials. The sample of the silk cotton used for the present studies was found to have (Figures expressed in percentage); N, 0.30; free-lipids, 1.40; OAc, 14.00; OMe, 4.40. The nature of the low percentage of nitrogen has not been investigated so far, although the nature of the polysaccharides has been reported by Timell *et al.*<sup>3,4</sup>. These workers have shown that the hemicellulose portion is a partially O-acetylated (8%), 4-O-methyl-glucurono-xylan<sup>3</sup>. The present report gives an account of the nature of the nitrogen present in the silk cotton.

Silk cotton, freed from free-lipids, (Ca., 300 mg), was hydrolyzed with 6 N hydrochloric acid (100 ml) in a sealed tube at 110° C for 30 hours<sup>5</sup>. The excess of the acid in the hydrolyzate was removed by repeated evaporation under reduced pressure. The residue was taken up in water (30 ml) and filtered. The filtrate was again evaporated to dryness and the residue dissolved in 10% propanol (1 ml). This was subjected to two-dimensional paper chromatography according to the procedure of Levy and Chung<sup>6</sup>, using Whatman No. 1 filter-paper with *n*-butanol : acetic acid : water, 4 : 1 : 5 v/v; and phenol : *m*-cresol : pH 9.3 borate buffer, 25 : 25 : 7 w/w/v, as solvent systems. Ninhydrin spray revealed, in decreasing order of concentration, the presence of glycine, alanine, serine, phenylalanine, valine, cysteine, glutamic acid and aspartic acid. In addition to these, trace amounts of 2 to 3 ninhydrin positive spots could be seen on the chromatograms. However these were not further investigated.

The notable feature of the silk cotton is the presence of high proportions of glycine followed by alanine, serine, and phenylalanine. This is reminiscent of the amino acid composition of natural silk which is mainly composed of glycine, serine, alanine and tyrosine. In fact in spite of the low nitrogen content (0.30%, which corresponds

to about 1.88% protein), silk cotton has the lustre and feel of natural silk. However, these physical characteristics could also be partly due to the high OAc (14%) and free-lipids associated with the silk cotton.

It is interesting to note the presence of S-containing amino acid and acidic amino acids, glutamic and aspartic acids. These amino acids along with arginine are the chief constituents of wool. In fact, silk cotton exhibits resilience which is a characteristic property of wool. Thus silk cotton imbibes, atleast partly, the physical properties of both natural silk and wool, and this behaviour may be due to its peculiar amino acid composition.

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## AMINO ACIDS OF SOME WILD LEGUMINOUS SEEDS

### ABSTRACT

Free amino acid composition of seven wild leguminous seeds has been studied. Each seed has been found to have its own pattern. Seed proteins have evinced the presence of all essential amino acids required for nutrition.

### INTRODUCTION

SINCE legumes in general form one of the most important sources of proteins in Indian dietary<sup>1,2</sup> many leguminous plants are cultivated throughout the country. Besides these, India abounds in many wild uncultivated leguminous plants which grow luxuriantly throughout the year due to favourable soil and climatic conditions<sup>3,4</sup>. Investigations<sup>5-10</sup> on several wild leguminous seeds indicated that they were fairly good sources of proteins and suggested their possible inclusion in animal nutrition. In view of this it was considered worthwhile to investigate their adequacy-both qualitatively as well as quantitatively-as complete proteins with

TABLE I  
Amino acid composition of some wild leguminous seed protein hydrolysates  
(Expressed as mg/100 mg defatted seed powder)

Amino acid	Seeds*						
	1	2	3	4	5	6	7
$\alpha$ -alanine	.. 3.51	2.42	1.09	0.79 (3.7)	2.76	3.19	2.34
Arginine	.. 3.40	3.09	2.43	2.02 (12.0)	3.14	3.00	2.82
Aspartic acid	.. 11.14	20.33	14.81	11.95 (8.3)	17.46	11.23	12.26
Glutamic acid	.. 7.85	18.20	12.75	11.15 (21.8)	2.22	9.44	6.54
Glycine-Serine	.. 10.85	15.26	7.37	5.74	7.49	7.31	7.63
Histidine	.. 2.37	2.80	1.90	1.34 (2.2)	1.56	1.77	2.13
Isoleucine-Leucine	.. 4.45	2.85	1.70	1.80 (9.6)	2.86	2.13	2.45
Lysine	.. 3.83	4.83	3.04	2.09 (4.5)	5.01	2.01	2.45
Methionine	.. 0.40	0.53	0.45	0.07 (1.1)	0.22	0.53	0.27
Phenylalanine	.. 0.85	1.06	0.73	0.29 (3.9)	1.68	0.90	1.57
Proline**	.. 6.14	4.83	4.09	1.37 (3.6)	5.24	3.87	4.00
Threonine	.. 2.08	5.12	3.76	1.88 (2.6)	0.95	0.41	1.44
Tryptophan	.. +	+	+	+	+	+	+
Tyrosine	.. 1.08	0.53	0.28	0.18	0.54	0.43	0.48
Valine	.. 2.20	1.88	1.21	0.36 (4.2)	1.11	0.84	1.17

\*\* Optical density measured at 440 m $\mu$ .

+ = Present, quantity not determined.

\* Seeds 1. *Bauhinia purpurea*, 2. *Cassia glauca*, 3. *Delonix regia* (red flowered), 4. *Delonix regia* (yellow flowered), 5. *Pongamia pinnata*, 6. *Prosopis juliflora*, 7. *Sesbania grandiflora*.

respect to their essential amino acid content. The present communication describes the analysis of seven uncultivated leguminous seeds of *Bauhinia purpurea*, *Cassia glauca*, *Delonix regia*, *Pongamia pinnata*, *Prosopis juliflora* and *Sesbania grandiflora* for free amino acids as well as for protein bound ones.

#### MATERIALS AND METHODS

Healthy and dry mature seeds collected locally and botanically identified were powdered to 100 mesh. They were defatted with petroleum ether (B.P. 40–60°C) and were preserved in dry air-tight bottles.

Free amino acids, both qualitative as well as quantitative for the protein bound ones were assayed in ethanolic extracts (70%, v/v) of the defatted seed powders. In some cases (*Bauhinia purpurea*, *Pongamia pinnata* and *Sesbania grandiflora*) the extracts were prepared in 90% (v/v) ethanol as these extracts gave better resolution of amino acids on the chromatograms.

For qualitative analysis of free amino acids, paper partition chromatographic technique<sup>11,12</sup> was employed. Amino acids were identified by employing the various special spray reagents<sup>13-19</sup>. Total free amino acids were estimated by the method of Rosen<sup>20</sup>.

Quantitative estimations of protein bound amino acids were made in the defatted seed powders from which all the free amino acids were removed by repeated extraction with aqueous ethanol (70%, v/v) till the washings became negative to ninhydrin test.

About 100 mg (accurately weighed) of this dry free amino acid-free powder of each seed were subjected to hydrolysis with hydrochloric acid (5 ml, 6N) in a sealed tube for 24 hours in an air oven (100–110°C) till the hydrolysate was negative to biuret test. The acid-free hydrolysate after repeated distillation *in vacuo* was subjected to chromatographic analysis and identification. The quantitative determination of protein-bound

amino acids was made by the procedure of Rosen<sup>20</sup> and expressed in terms of glycine. Tryptophan was detected in the alkaline hydrolysate of the seed powder.

The free amino acids of the wild leguminous seeds were determined and it was found that each seed has its own amino acid pattern. In general,  $\alpha$ -amino-isobutyric acid, lysine and tyrosine could not be detected in most of the seeds. Other amino acids seem to be present in varying amounts. The contents of the total free amino acids (expressed as mg glycine/100 mg defatted seed powder) are as follows: (1) *Bauhinia purpurea*, 0.290; (2) *Cassia glauca*, 0.133; (3) *Delonix regia*, (red flowered) 0.266; (4) *Delonix regia* (yellow flowered), 0.317; (5) *Pongamia pinnata*, 1.120; (6) *Prosopis juliflora*, 0.464; (7) *Sesbania grandiflora*, 0.93.

One of the reasons for the variation in the total free amino acid content could be due to the various stages of maturity of the seeds at the time of their collection and also to the rate of protein synthesis during seed ripening. All the seeds analysed except those of *Bauhinia purpurea* and *Cassia glauca* reveal the presence of one unidentified ninhydrin positive compound. Attempts are being made to isolate and characterize them.

Results of the protein-bound amino acid analysis (Table I) reveal that all the seeds contain practically all the essential amino acids although not in adequate quantities as recommended by FAO. However, it is noteworthy that protein hydrolysates of all the seeds show appreciably high concentration of aspartic and glutamic acids. *Bauhinia purpurea* shows a higher concentration of proline than any other seed.

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### EFFECT OF *PSEUDOMONAS GLYCINIA* EXOTOXIN ON CHEMICAL COMPOSITION OF HOST LEAVES

*Pseudomonas glycinea* which causes haloblight in soybean also produces a chlorosis-inducing exotoxin<sup>1</sup>. The exotoxin has been reported to be a polysaccharide of low molecular weight and identical to exotoxin of *Pseudomonas phaseolicola*<sup>2,3</sup>. It induces chlorosis starting from veins ultimately extending to cover full leaf within two to three days. The mode of action of this exotoxin is not known. However, the exotoxin increased the ribonuclease activity as well as the rate of uracil 2-C-14 incorporation into RNA fraction of host leaves<sup>4</sup>. In the present communication the influences of this exotoxin on chemical composition of host leaves has been studied.

The methods for the estimation of chlorophylls *a* and *b*<sup>5</sup>, DNA and RNA<sup>6</sup>, protein<sup>7</sup>, free amino acids<sup>8</sup>, water soluble sugars<sup>7</sup> and reducing and non-reducing sugars<sup>9</sup> were as described. The exotoxin was extracted and partially purified from culture filtrate of *Pseudomonas glycinea* with slight modification of the method of Hoitink and Sinden<sup>2</sup>. Instead of the bacterial filters, chloroform was used to kill the bacteria and the step of thin layer chromatography for purification was omitted. To obtain exotoxin affected leaves, month old plants were placed in nutrient medium containing exotoxin. Control plants were placed in nutrient medium without the toxin. Symptoms appeared within three to four days at first in lower leaves.

The data summarized in Table I indicate that exotoxin treatment had decreased both chlorophylls *a* and *b*. However, the decrease was much greater for chlorophyll-*b* than for chlorophyll-*a*. In fact only 14% of chlorophyll-*a* and 6% of chlorophyll-*b* of that present in healthy leaves remained in the treated leaves. The affected leaves contained less DNA but more RNA compared with the control. Free amino acids accumulated perhaps at the



TABLE I

Effect of exotoxin of *pseudomonas glycinia* on the chemical composition of soybean leaves

Sl. No.	Name of the constituents	Healthy leaves	Exotoxin affected leaves
		mg/100 gm dry leaves	
1.	Chlorophyll- <i>a</i>	0.213	0.035
2.	Chlorophyll- <i>b</i>	0.150	50.009
3.	DNA*	15.15	10.1
4.	RNA*	111.00	167.00
5.	Free amino acids	26.9	50.00
		g/100 dry leaves	
6.	Protein	28.44	25.93
7.	Reducing sugars	1.650	2.057
8.	Non-reducing sugars	0.463	0.400
9.	Total of reducing and non-reducing sugars	2.113	2.457
10.	Water soluble carbohydrates	4.407	5.254

\* Estimations were conducted in fresh samples but reported on dry weight basis.

expense of proteins. Total sugars and water soluble carbohydrates also increased in treated leaves indicating that the rate of utilization of some soluble polysaccharide is decreased. The possibility of accumulation of these sugars due to high rate of their synthesis is not there because the exotoxin treated leaves were chlorotic. Reducing sugars had also increased but non-reducing sugars had slightly decreased by treatment with exotoxin.

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#### ON THE INCIDENCE AND EFFECTS OF INFESTATION OF *SELENOTHrips RUBROCINCTUS* (GIARD) (THYSANOPTERA : HELIOTHIRIPINAE) ON THE FREE AMINO-ACIDS OF SOME SUSCEPTIBLE HOST PLANTS

THE increasing incidence of *Selenothrips rubrocinctus* the red-banded thrips, the notorious cacao and cashew pest of the West Indies in this country would appear to be of particular interest in view of the recent discovery of infestations of this species on very young plants of *Mangifera indica*, *Eugenia jambos*, *Psidium guava* and *Anacardium occidentale* from Kerala. First recorded by Moulton<sup>1</sup> in 1928 as stray individuals on flowers of *Aporosa* and *Cardenia* in Calcutta and subsequently on cashew in 1931 by Ramakrishna and Margabandhu<sup>2</sup>, this species was noticed in considerable numbers on wild species of *Jatropha*, a common hedge plant all over Kerala in 1964 by Ananthakrishnan. The sudden spread of this species which interestingly enough has taken to some of its typical host plants in Trinidad, like guava, *Terminalia*, cashew, mango (Williams, 1918<sup>3</sup>), can stand comparison with *Retithrips syriacus* (Mayet) first recorded by Seshadri and Ananthakrishnan<sup>4</sup> (1953) in this country and which since then extended its activity to no less than 25 hosts, some susceptible, others tolerant to attack. In view of grapevine, *Terminalia catappa* and *Eugenia jambolana* also being known hosts in Trinidad, the prospects of *Selenothrips* competing with *Rhipiphorotherips cruentatus* and *Retithrips syriacus* for such hosts cannot be ignored. Alongside with *Caliothrips indicus* (Bagnall) and the above two species, *Selenothrips* can easily be expected to occupy the status of a serious polyphagous pest. Further, in view of the confirmed occurrence of this species on host plants in different parts of Kerala, combined with the tendency of this species to breed prolifically on the leaves of its wild host suggests the possibility of its migration to other hosts. The nymphs of this species can at once be recognized by the bright red band across the body, while the adults possess a highly polygonally reticulate body with needle-like terminal antennal joints and broad wings with dark strong, stiff setae.

Group feeding of 30-40 adults and larvae per leaf was evident on young leaves of growing mango seedlings, while in *Eugenia jambos* and guava plants, they were more scattered resulting in feeding patches all over the leaves. Serious infestation leading to several types of feeding patterns as described by Fennah<sup>5</sup> (1964) in Trinidad on cashew leaves were not observed, though the nymphs showed the commonest distribution pattern belonging to the basal primary subvascular type, i.e., grouped along the sides of the midrib basally, but the adults showed a random distribution.

Infested leaves of plants susceptible to attack by thrips as in the case of other insects, tend to show an increase in the concentration and in some cases also the number of free aminoacids, as against the more tolerant plants like the brown stemmed variety of *Ricinus communis*, tolerant to *Retithrips syriacus* and where the number and concentration of the free aminoacids do not differ considerably in the infested and healthy leaves. Ananthakrishnan and Muraliedharan<sup>6</sup> (1972) also indicate that lower concentrations of some of the free aminoacids may evoke an aggregation response as against a feeding inhibition or host avoidance reaction, when in higher concentrations. However the deficiency in the number of free aminoacids and lower concentration of the available ones as seen in the more resistant plants are evidently not conducive to the growth and development of the concerned insects. The results herein presented relate to the susceptibility of the host plant concerned to the attack of *Selenothrips rubrocinctus* as judged by the relative increase in the number and concentration of free aminoacids in the infested leaves.

The free aminoacids were extracted from the infested and non-infested leaves (Ananthakrishnan and Muraliedharan, 1972) and qualitative determination made by using the descending monodimensional paper chromatography, the solvent used being a mixture of 120 ml of 2-Butanol with 40 ml of 3% Ammonium hydroxide (Roland and Gross<sup>7</sup>, 1954), the chromatograms run for 48 hours, dried and sprayed with 0.1% Ninhydrin.

Of the 4 host plants examined, which were susceptible to attack, only *Anacardium occidentale* showed the presence in the severely attacked leaves of extra free aminoacids cystine, lysine-histidine, serine-glycine-aspartic acid, not normally present in the non-infested leaves of the same age and more concentration of glutamic acid-threonine, proline, alanine, valine-methione and leucine-isoleucine, indicating the high degree of preference and susceptibility of this host. These results favourably compare with those of Fennah (1964) who also reports the presence of other aminoacids such as

hydroxyproline, aminobutyric acid, etc. In some infested leaves he has also indicated the differences between the aminoacid content of the laminal tissue close to the midrib and that near the edge of the leaf, in healthy and infested leaves, showing an increase in aminoacid content of attacked leaves. The infested leaves of all the other three host plants discussed here were not observed to show the presence of any extra free aminoacids, but all the same the concentrations of their free aminoacids, as compared with those of non-infested leaves were heavy, lending sufficient justification to consider these hosts as equally susceptible to attack, though second only to *Anacardium*. A comparative picture of the free aminoacids in the infested leaves of all the four host plants are provided in Table I from

TABLE I  
Free aminoacids of infested leaves

Free aminoacids	<i>Anacardium occidentale</i>	<i>Eugenia jambos</i>	<i>Mangifera indica</i>	<i>Psidium guava</i>
Cystine	++	++	-	-
Lysine-Histidine	++	++	++	+
Serine-Glycine-Aspartic acid	+++	++	++	++
Alanine	++	+++	++	++
Glutamic acid-Threonine	++	+++	++	++
Tyrosine	Tr.	+	++	Tr.
Valine-Methionine	++	++	++	++
Leucine-Isoleucine	++	-	-	++
Proline	++	+++	+++	+

which it may be observed that the presence in higher concentrations of glutamic acid-threonine, valine-methionine, alanine, serine-aspartic acid-glycine, lysine-histidine and proline appear to be a uniform feature in all the susceptible plants resulting from *Selenothrips* infestation, though the presence of other aminoacids may be variable both in number and concentration.

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**POST-INFECTION CHANGES IN ASCORBIC  
ACID CONTENTS OF 'AONLA'  
(*PHYLLANTHUS EMBLICA* L.) FRUITS CAUSED  
BY *ASPERGILLUS NIGER* VAN TIEGH.**

'AONLA' has aroused good deal of interest among the Scientific workers because it is one of the richest natural source of Vitamin C (ascorbic acid). Biosynthesis of ascorbic acid in plants has been extensively reviewed by Mapson<sup>1</sup> as well as Isherwood and Mapson<sup>2</sup>. Recently many investigators have estimated the changes in quantities of ascorbic acid in infected fruits. It is, therefore, considered desirable to study the changes in vitamin C contents of 'aonla' fruits under pathogenesis.

The healthy fruits of 'aonla' of nearly the same age were inoculated with a single loopful of the spore suspension of *A. niger* containing about 100 spores per high power field of the microscope. The

healthy fruits inoculated with the same amount of sterile distilled water served as the control. Both types of fruits were incubated at  $25 \pm 2^\circ \text{C}$  for 14 days. On every alternate day, the extracts from healthy and diseased fruit tissues were prepared by crushing 5 g of the fruit tissues in 25 ml of 5% metaphosphoric acid in a ground glass homogenizer and the contents were filtered. The residue was washed twice with 10 ml of 5% metaphosphoric acid and the total volume of the filtrate was finally raised to 50 ml by adding the required amount of metaphosphoric acid. 10 ml of the filtrate was titrated against previously standardized solution of 2-6-dichlorophenol indophenol reagent recommended by Bessey and King<sup>3</sup>. The quantities of ascorbic acid in mg/100 g of the fruit tissue were calculated. The results are summarized in Table I, where the percentage decay on different days is indicated in brackets.

It is evident from Table I that with an increase in the incubation period there was a gradual decrease in the ascorbic acid contents of healthy as well as infected fruits of 'aonla'. The percentage losses in infected fruits was 84.2 after 14 days of incubation while corresponding loss in healthy fruits was only 41.1. Similar decline in ascorbic acid was obtained by Singh and Tandon<sup>4</sup> in Guava fruits infected with *Aspergillus niger*. It is evident, from the present investigation, that the losses in ascorbic acid contents were directly proportional to the percentage of rot in the fruits.

Comparatively rapid decline in ascorbic acid in the infected tissue may be due to increased respiration rate under pathogenesis as observed in many fungi including powdery mildews and rusts, etc., by Samborski and Shaw<sup>5</sup>, Daly *et al.*<sup>6</sup> as well as Bunshnell and Allen<sup>7</sup>. The decline in ascorbic acid may also be due to the production of suitable

TABLE I  
Post-infection changes in ascorbic acid content (in mg/100 g in the pulp) of Healthy and infected 'aonla' fruits

Fruits	Ascorbic Acid contents								% loss in ascorbic acid after 14 days of incubation
	Days of incubation								
	0	2	4	6	8	10	12	14	
Healthy (Control)	.. 570.2	528.8	496.7	459.2	428.1	388.5	353.7	335.5	41.1
		(No decay in any case)							
Infected	.. ..	495.2	424.1	384.2	339.31	212.5	140.32	90.1	84.2
	.. ..	(1.4)	(5.2)	(12.1)	(26.5)	(41.5)	(64.5)	(84.2)	

ascorbic acid degrading enzymes either by the pathogen or by host pathogen interactions.

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## STUDIES ON THE ROSETTE VIRUS INFECTED GROUNDNUT (*ARACHIS HYPOGAEA* L.)

### I. Effect on Free Amino Acid

ROSETTE is a virus disease of groundnut (*Arachis hypogaea* L.) transmitted in the field by *Aphis cracivora* Koch<sup>1</sup>. Rosette virus produces severe mosaic mottling, reduction of leaf, stunting of the

plant and almost total loss of yield, if the plant is infected at an early date. Several workers have studied the changes in free amino acids caused by viruses<sup>2-4</sup> but the relationship of symptom severity with free amino acid changes in virus infected plants is not clear. Virus diseases like symptoms have been induced by supplying excess concentration of certain amino acids to a healthy plant<sup>3,5</sup>. However, information on the free amino acid contents of rosette virus infected groundnut plant is not worked out. The purpose of this study is to (1) determine the changes in free amino acid concentration, induced by rosette virus at the time of different phases of symptoms, (2) correlate symptom severity with free amino acid concentration. The correlation among symptom severity, and free amino acid concentration changes was investigated by using rosette virus that caused different manifestation of symptom in groundnut plants.

Groundnut plants (local variety) infected under field conditions by rosette virus were used for present study. Leaves of same age exhibiting different degrees of symptoms were grouped as mild mosaic (MM), mosaic with slight reduction (MR) and severe reduction (SR), as compared to healthy. In addition to leaf samples the stem and root were also analysed for amino acid. Composite samples from the infected plants showing different phases of symptoms in leaves along with stem and root were collected. Similarly the leaf, stem and root

TABLE I

Quantity of free amino acids (mg./100 mg dry weight) in rosette virus infected groundnut plant parts

Amino acid		Leaf				Stem		Root	
		H	MM	MR	SR	H	D	H	D
Glutamine	..	0.12	0.42	0.42	0.82	0.18	0.77	0.14	0.07
Aspartic acid	..	0.13	0.13	0.10	0.30	0.08	0.13	0.07	0.14
Glutamic acid	..	0.13	0.24	0.34	0.34	0.18	0.26	0.02	0.38
Alanine	..	0.30	0.40	0.54	0.60	0.16	0.18	0.15	0.20
Proline	..	0.20	0.16	0.14	0.03	0.15	0.12	0.10	0.08
Tyrosine	..	0.48	0.50	0.44	0.40	0.14	0.20	0.08	0.20
Tryptophan	..	0.14	0.16	0.13	0.09	0.05	0.09	0.05	0.14
Phenyl alanine	..	0.04	0.01	0.01	0.01	0.02	0.01	0.01	0.03
Leucine	..	0.07	0.10	0.10	0.13	0.04	0.02	0.02	0.06
Total	..	1.61	2.12	2.22	2.72	1.20	1.78	0.64	1.30

H = Healthy, MM = Mild mosaic, MR = Mosaic with slight reduction, SR = Severe reduction, D = Diseased, \* Significant at 5% level.

samples were also collected from the healthy groundnut plants of the same age. Samples for free amino acid estimation were prepared and analysed by two-dimensional paper chromatography<sup>6</sup> using Whatman No. 1 chromatographic paper. The first solvent phase was *n*-butanol, acetic acid and water (12:3:5) and second solvent phase was phenol-water (80:20). The quantity of amino acid was estimated by comparing their transmission with their respective amino acid standard curves prepared by graded concentration of each amino acid by using Densitometer type CM 11/5.

Results of the table indicate that each sample of groundnut plants contained 9 free amino-acids and there was no qualitative difference between healthy and diseased plant parts. The variation in the amount of each amino acid was evident. The concentration of total amino acids was increased in diseased plant than healthy. In the diseased samples, the highest amount of free amino acid concentration was in the SR followed by MR, MM, stem and root. Among the leaf samples the accumulation of aspartic acid, alanine, leucine and glutamine was maximum in SR. Tyrosine and tryptophan content of MR and SR were slightly lower compared to the healthy leaves and MM but the quantities were slightly higher in MM than in the healthy leaves. Proline and phenyl alanine was higher in healthy leaf and stem than diseased but former is higher in healthy root whereas latter in diseased root.

The present finding revealed that the free amino acid content appeared closely correlated with symptom severity. Free amino acid concentration increase was in order of SR > MR > MM rosette virus infected groundnut leaves. Commoner and Nehari<sup>3</sup> suggested that virus disease symptoms might result from free amino acid and amide increase or decrease in infected plants. Tu and Ford<sup>7</sup> also observed a correlation between free amino acid and soybean infected, with 3 isolates of soybean mosaic virus. An increase in aspartic acid, alanine, leucine and glutamine was also observed in several virus infected plants like<sup>7-11</sup> our findings. With many virus diseases, an accumulation of free amino acid in diseased plants parts has been reported.<sup>11-13</sup>

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#### STUDIES ON THE FEEDING HABITS OF *ERETES STICTICUS* (L.) (DYTISCIDAE COLEOPTERA)

In a study on the systematics and ecology of aquatic coleoptera of Visakhapatnam area, it has been found that the adult dytiscid *Eretes sticticus* (L.) mostly feeds on the larvae of mosquitoes *Anopheles* and *Culex*. This preference in feeding habits has stimulated our interest to take a close look. The insect is nocturnal occurring throughout the year in ponds and pools devoid of vegetation. They are always gregarious and occur in societies in ponds (where oxygen content was even 2 ml/ltr.) and semi-dry, swampy, humid regions. It is of interest to note that *E. sticticus*, recorded earlier in India<sup>1</sup> and in Ceylon<sup>2</sup>, is present in large numbers in Visakhapatnam.

A perusal of the literature shows that *E. sticticus* is the only coleopteran known to show selectivity in food. To assess the degree of selectivity shown by the insect, experiments were conducted in the laboratory with males and females before and after starvation (for a period of 4 days) and offering a variety of prey animals like mosquito larvae, chironomids, other insect larvae (*Anagrus robusta*, *Polymitaeris* sp.) and dead fishes (*Apocheilus melastigma*). In the case of females selectivity and rate of feeding have been recorded before and after oviposition (Table I). Experiments were also conducted to assess the importance of *E. sticticus* as a potential control measure for mosquito larvae and to determine the critical stages of the mosquito larvae which are more vulnerable to attack by *E. sticticus* (Table II). These experiments were repeated four times and the average values are presented.

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TABLE I

*Preferential feeding of E. sticticus on a mixed diet (mosquito larvae, chironomid larvae, other insect larvae, and dead fish) expressed as weight in milligrams during a period of 12 hours*

Expt. No.	Mosquito larvae (15 mg)	Chironomid larvae (25 mg)	Other insect larvae ( <i>Anagrus robusta</i> , <i>Polymita</i> spp.) (32 mg)	Dead fish ( <i>Aplocheilum melastigma</i> ) (1500 mg)
1. Male (unstarved)	15 mg	15 mg	25.6 mg	550 mg
2. Female (unstarved)	15 mg	12.5 mg	12.8 mg	250 mg
3. Male (after starvation)	15 mg	25 mg	32 mg	950 mg
4. Female (after starvation)	15 mg	20 mg	28.8 mg	850 mg
5. Female (before oviposition)	15 mg	..	..	..
6. Female (after oviposition)	15 mg	22.5 gm.	32 mg	450 mg

TABLE II

*Preferential feeding of E. sticticus on mosquito larvae expressed as percentages of total number of larvae offered as diet during a period of 1 hour*

Expt. No.	I Instar	II Instar	IV Instar	Pupae
1. Male (unstarved)	25 %	20.8 %	25 %	8.3 %
2. Female (unstarved)	25 %	16.6 %	16.6 %	4.1 %
3. Male (after starvation)	25 %	25 %	25 %	16.6 %
4. Female (after starvation)	25 %	20.8 %	25 %	8.3 %
5. Female (before oviposition)	8.3 %	4.1 %	..	..
6. Female (after oviposition)	25 %	25 %	16.6 %	8.3 %

When a mixed diet comprising the organisms mentioned above is offered, first the mosquito larvae were attacked followed by chironomids, other insect larvae and dead fish in that order. Males of *E. sticticus* appear to be voracious feeders in comparison to females (Table I). But females generally feed faster after oviposition because they cease feeding before oviposition for nearly two days. However, both males and females resort to faster feeding after starvation. The I (8.3%–25%), II (4.1%–25%) and IV (16.6%–25%) instars of the mosquito larvae bore the brunt of predation more severely than the pupae (4.1%–16.6%).

It may be mentioned that it is only in the males that the front pair of legs possess tarsal dilations with cupules (Fig. 1) with which they hold and lift the prey with ease during foraging activities. A united attack on the prey by several insects is observed frequently in the laboratory. This species being agile during night, the rate of feeding is also higher during the night than during the day.



FIG. 1. The front leg of male *E. sticticus* showing tarsal dilation with cupule.

Judged from the results of our experiments it could be said that *E. sticticus* is an efficient

predator on mosquito larvae. The possibility that it could serve as a powerful biological control agent has to be explored further.

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### FEATURES OF TAXONOMIC VALUE IN *HYALOMMA MARGINATUM ISAACI* SHARIF, 1928\*

#### ABSTRACT

The percentage loss in weight during pre-oviposition and at the end of ovipositional periods in *Hyalomma marginatum isaaci* was observed to be  $16 \pm 4.73$  and  $85 \pm 0.81$ , respectively. The pre-oviposition and ovipositional periods at a max  $25^{\circ}\text{C} - 14^{\circ}\text{C}$  were 6-9 days and 24-30 days, respectively. For every milligram increase in the body weight a yield of 7.0786 (7) eggs was noticed. It was observed that an average weight loss of 85% during the course of egg-laying and an average weight gain of 67.7% in respect of egg production, resulting in an average actual loss of 17.3%. The potentiality of conversion of imbibed materials into eggs and the yield of eggs for every milligram increase in body weight are emphasised in tick taxonomy.

MODERN researchers consider many other features important in the characterisation of species of ticks in addition to morphology. Nagar (1968a) made an attempt to show that the ovipositional rate in *Dermacentor variabilis* and *Rhipicephalus sanguineus* has some value in the taxonomy of ixodid ticks. The present paper records a similar observation made on *Hyalomma marginatum isaaci* which has been recently incriminated as the transmitter of Kyasanur Forest Disease virus transstadially (Singh and Bhat, 1968).

**Materials and Methods.**—Engorged female ticks of *Hyalomma marginatum isaaci* were collected from the body of sheep belonging to the Sheep Breeding Farm, Dhanagur, Mandya District, Karnataka State. They were weighed in a mettler balance and the weight was referred as 'initial weight'. Ticks were kept in petri-dishes individually with filter-paper inside for oviposition under laboratory conditions at max  $25^{\circ}\text{C} - \text{min } 14^{\circ}\text{C}$ . The weight of the tick was recorded on the day before laying first egg and referred as 'ovipositional weight'. Every morning

the number of eggs laid by a tick were counted under a dissecting microscope and weighed. The final weight of each tick was noted at the cessation of egg-laying and referred as 'postovipositional weight'. The duration of the pre-oviposition and ovipositional periods were also recorded.

**Results.**—The initial weight range was between 561 to 1106 mgms. The initial weight, the ovipositional weight, the percentage loss in the weight during pre-oviposition period and its duration and number of eggs laid by each tick is presented (Table I). The percentage loss in weight during pre-oviposition period is  $16 \pm 4.73$ . Similarly, the percentage loss occurred during the oviposition is also calculated and presented in Table II. The ticks lost  $85 \pm 0.81\%$  of their initial weight during the course of egg-laying.

The range of pre-oviposition and ovipositional periods was between 6-9 days (average 7.8 days) and 24-30 days (average 27.3 days), respectively.

Tables I and II indicate that the ticks with lighter initial weight laid smaller number of eggs. When values were plotted, a linear relationship found (Fig. 1). Linear regression analysis made

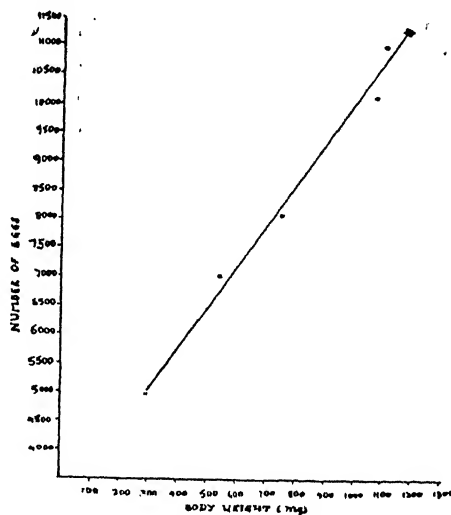


FIG. 1. *Hyalomma marginatum isaaci*: Linear relationship between body weight and number of eggs.

resulted in a formula as follows:  $N = 2820.5 + 7.0786 W$ . ( $N$  = number of eggs,  $W$  = weight of the tick). The linear regression analysis between the weight of tick and the number of eggs never become zero at a particular weight of the tick. The equation indicates that for every milligram increase in the body weight 7.0786 (7) eggs were produced.

Table III shows an average weight loss of 85% during the course of egg-laying and an average

TABLE I

Percentage loss in the weight during pre-oviposition period

Tick No.	Initial weight (mgm)	Ovipositional weight (mgm)	Percentage loss in weight	Pre-oviposition period (days)	Total number of eggs
1 H	561	460.1	18.0	6	6919
2 H	760	649.9	14.4	9	8073
3 H	1106	870.5	21.2	8	11030
4 H	1080	970.2	10.2	8	10161

Mean loss — 16% Standard deviation — 4.73

TABLE II

Percentage loss in the weight at the end of oviposition period

Tick No.	Initial weight (mgm)	Postovipositional weight (mgm)	Percentage loss (mgm)	Oviposition period (days)	Total number of Eggs
1 H	561	90.2	84	24	6919
2 H	760	110.8	85	27	8073
3 H	1106	170.8	65	28	11030
4 H	1080	150.5	86	30	10161

Mean loss — 85% Standard deviation — 0.81

TABLE III

Percentage of weight gain in terms of egg yield

Tick No.	Initial weight (mgm)	Post-ovipositional weight (mgm)	Actual loss in weight (mgm)	Weight of eggs (mgm)
1 H	561	90.2	470.8 (84%)	436.4 (77.8%)
2 H	760	110.8	649.2 (85%)	500.4 (65.8%)
3 H	1106	170.5	935.5 (85%)	658.7 (59.6%)
4 H	1080	150.5	929.5 (86%)	729.2 (67.5%)

Average weight Loss — 85%, Average weight gain in terms of eggs — 67.7%, Average actual loss — 17.3%.

weight gain 67.7% in terms of egg production resulting in an average actual loss of 17.3%.

**Discussion.**—The pre-oviposition and ovipositional periods in *Hyalomma marginatum isaaci* at 28–32°C was observed to be 3–6 days and 18–19 days, respectively (Jagannath *et al.*, 1972), while

Das and Subramanian (1971) recorded it to be 8–10 days and 16–19 days, respectively, at 27.8–29.8°C. In the present study these periods were found to be 6–9 days and 24–30 days, respectively, at 14–25°C. In view of these variations, it is evident that pre-oviposition and ovipositional periods bear a direct relationship to temperature changes (Nagar, 1968 b).

The percentage loss in the weight during the pre-oviposition period was different for all the four ticks though they were kept under identical conditions whereas Nagar (1968 b) found the percentage weight loss in *Rhipicephalus sanguineus* and *Dermacentor variabilis* to be approximately the same. The metabolic rate of individual ticks might vary and could account for the difference in percentage weight loss.

Table III shows that the total loss at the end of ovipositional period to be 85% and the weight gain in terms of egg yield was 67.7%. The actual loss in weight which was 17.3% (barring the weight of the dead tick) might have been due to loss of excreta and a part of the body reserve spent on the conversion of imbibed blood into eggs. The average gain in terms of egg yield to the tune of 67.7% of the initial weight shows its ability to convert the imbibed material into eggs. The ability to convert food material to eggs, if differs from species to species barring individual variations might be of some taxonomical value.

The linear relationship between the body weight and the number of eggs laid by *Hyalomma marginatum isaaci* was similar to the observations made by Kitaoka and Yajima, 1958; Achan, 1961; Snow and Arthur, 1966 and Nagar, 1968 a. For every milligram increase of the body weight the yield was 7.0786 eggs while it was 13.939 eggs in *R. sanguineus* (Nagar, 1968 a); 11.9 eggs in *Boophilus caudatus*, 10.8 eggs in *Haemaphysalis campanulata*; 10.2 eggs in *H. bispinosa* (Kitaoka and Yajima, 1958); 9–10 eggs in *Hyalomma anatolicum anatolicum* (Snow and Arthur, 1966) and 7.733 eggs in *D. variabilis* (Nagar, 1968 a). If the capacity of a tick in laying a certain number of eggs per milligram body weight is found to be useful taxonomic feature as presumed by Snow and Arthur (1966) and Nagar (1966 a), the present egg yield noticed in *Hyalomma marginatum isaaci* for every milligram of increase in body weight could be a valuable information for speciation in ixodid ticks.

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#### ANATOMICAL OBSERVATIONS ON *MOLLUGO OPPOSITIFOLIA* L. WITH REFERENCE TO ITS ADAPTIVE SIGNIFICANCE

*Mollugo oppositifolia* L. is found in basal parts of walls, on heaps of ruined houses and on alluvium deposits in Gorakhpur. In India *Mollugo hirta* Thunb<sup>1</sup>, *Mollugo nudicaulis* Lam.<sup>2</sup> and *Mollugo cerviana* Sers.<sup>3</sup> have been worked out from ecological point of view. The author during the eco-physiological studies of *Mollugo oppositifolia* noted certain unreported anatomical peculiarities which seem to be of great adaptive value for the species in its habitat.

Root and stem of the plants from the wall and alluvium soil were collected in October, 1972 from Hanumangarhi, Gorakhpur and fixed in F.A.A. After preparing double stained free hand and microtome sections using safranin and fast green stains anatomical studies were made.

The anatomical structure of root and stem earlier to secondary growth shows normal dicot. characters. However, after secondary growth there is found abnormality in origin and activity of accessory cambia in the species.

**Secondary growth in stem.**—It initiates by the appearance of a normal cambium ring which remains active for an indefinite period producing abundant xylem parenchyma accompanied by vessels and tracheids towards pith, and towards periphery it cuts off secondary phloem in which phloem parenchyma is highly developed (Fig. 1). Xylem parenchyma and phloem parenchyma work as storage tissue. After sometime, an abnormal accessory cambium originates in the peripheral part of the phloem tissue which cuts off secondary xylem and prosenchyma tissue towards pith and towards periphery it cuts off secondary phloem and prosenchyma tissue (Fig. 3). In succession thus three to four accessory cambia develop in the peripheral part of phloem tissue which behave like the first accessory cambium. Xylem parenchyma, phloem parenchyma and prosenchyma show interpenetration with conductive tissue and they work for storage of starch grains. One special feature in the material collected from wall habitat is that in early stages growth is of centric type where on one side the cambium shows poor activity. Later on secondary growth changes to excentric type due to the appearance of successive cambia on one side only (Fig. 2). The pericycle breaks at many points in advanced stage of secondary growth.

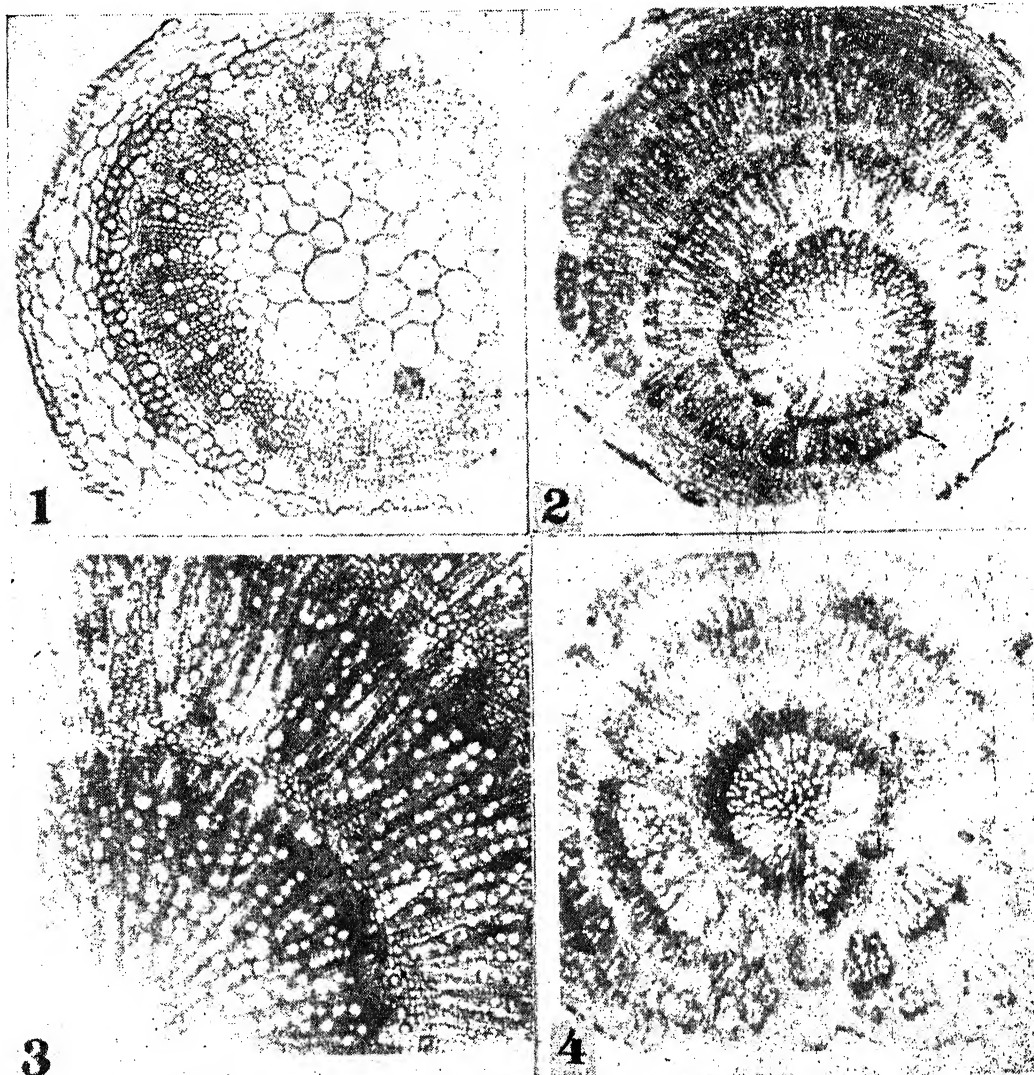
**Secondary growth in root.**—The cambium originates as in a typical dicot. root which cuts off secondary tissues for indefinite period resembling to the stem. Three to four accessory cambia originate in the peripheral part of phloem tissue in succession which cut off rings of xylem, phloem and prosenchyma tissue (Fig. 4). In the material collected from the wall, in the initial stage secondary growth is of centric type but in advanced stage it becomes excentric. However, in the material collected from alluvium soil it remains of centric type (Fig. 4).

Thus in root and stem there seems to be a general tendency for high development of storage tissues in different rings.

**Discussion.**—The upper part of *Mollugo oppositifolia* is commonly subjected to grazing. The basal part of such grazed shoots exhibits abnormal secondary growth. Accessory cambia originate also in the peripheral part of phloem tissue in succession which cut off good amount of storage and conductive tissues. There is interpenetration of storage and conductive tissues which renders the disposition and renewal of reserve food easier and more effective<sup>5</sup>. For regeneration of shoot after grazing and also to meet the requirement of food during exhaustive fruiting period this reserve food plays a significant role.

The anatomy of old root as shown in Fig. 4 reveals the presence of a bulk of secondary xylem arranged in rings which besides conduction also provides good mechanical support needed for the successful growth in hard substratum (Joshi and Kamboj)<sup>4</sup>.

Hence the two different habits are due to texture of soil. In horizontal roots due to gravitational force the concentration of hormone is more on the lower side and less on the upper side but in the upright roots hormones are uniformly distributed. The stem is procumbent or pendent in habit due



FIGS. 1-4. Fig. 1. T.S. young stem showing normal activity of cambium. Fig. 2. T.S. stem after secondary growth with three accessory cambial rings and excentric activity. Fig. 3. A part of old stem enlarged. Fig. 4. T.S. root after secondary growth with three rings of cambia.

In root and basal zone of stem and interesting feature is the development of concentric rings in initial stage and in advanced stage it becomes excentric, in the material collected from the wall. The root system on the wall due to horizontally placed bricks is maintained in horizontal position. In the alluvium soil it is observed to be vertical,

to which the upper exposed part is highly illuminated and the lower unexposed part is less illuminated. This results in the unequal distribution of hormones. Konning<sup>10</sup> reports the unequal distribution of hormones C<sup>14</sup> labelled I.A.A. in horizontal pea roots, with more below than above. Torry and Loomis<sup>8</sup> suggest that I.A.A. stimulates growth

in vascular cambium<sup>7,8</sup>. In *Mollugo oppositifolia* the horizontal position in the root and procumbent or pendent habit in shoot results in unequal distribution of hormones which may be the cause of excentric growth in the material.

Metcalfe and Chalk<sup>6</sup> have classified the anomaly in the stems of Ficoidae in three groups: (1) Numerous bundles arranged in more or less distinct concentric bundles embedded in prosenchyma tissue. (2) Alternating more or less complete rings of xylem and phloem. (3) Transition between one and two. The abnormality of *Mollugo oppositifolia* is of the second type.

The author is greatly indebted to Principal Dr. Y. B. Singh and Dr. G. C. Srivastava, Head, Botany Department, for encouragements. The author further expresses his deep sense of gratitude to Dr. K. C. Misra, Reader, Botany Department, B.H.U., for valuable suggestions and to Dr. S. N. Dixit, Reader, Botany Department, Gorakhpur University, for going through the manuscript. Thanks are also due to Shri J. Abraham, Dr. A. B. Sinha and Shri A. K. Srivastava for their help and cooperation.

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## CONTRIBUTIONS TO THE FLORAL ANATOMY OF LINACEAE 3

### INTRODUCTION

THE floral anatomy of only a few taxa of Linaceae has been studied in the past (Narayana, 1964; Narayana and Rao, 1966, 1969, 1971). The present paper deals with the floral anatomy of *Sarcotheca glauca* Hall and *Sarcotheca oblongifolia* Merrill.

### OBSERVATIONS

**Flower.**—The flower is pedicellate, pentacyclic, pentamerous, heterochlamydeous, regular, bisexual and hypogynous. The sepals and petals show quincuncial and contorted aestivation respectively

(Figs. 5, 6, 7). The ten stamens are united to form a tube at the base (Fig. 7). They are of two lengths the antipetalous members being shorter. The anthers are dorsifixed and introrse. The gynoecium is 5-carpellary, syncarpous, 5-locular with two superposed, anatropous, bitegmic ovules in each loculus (Figs. 1, 8). The epidermal cells lining the loculi are thin-walled and tangentially flattened. The styles are free with capitate stigmas bearing glandular hairs (Fig. 1).

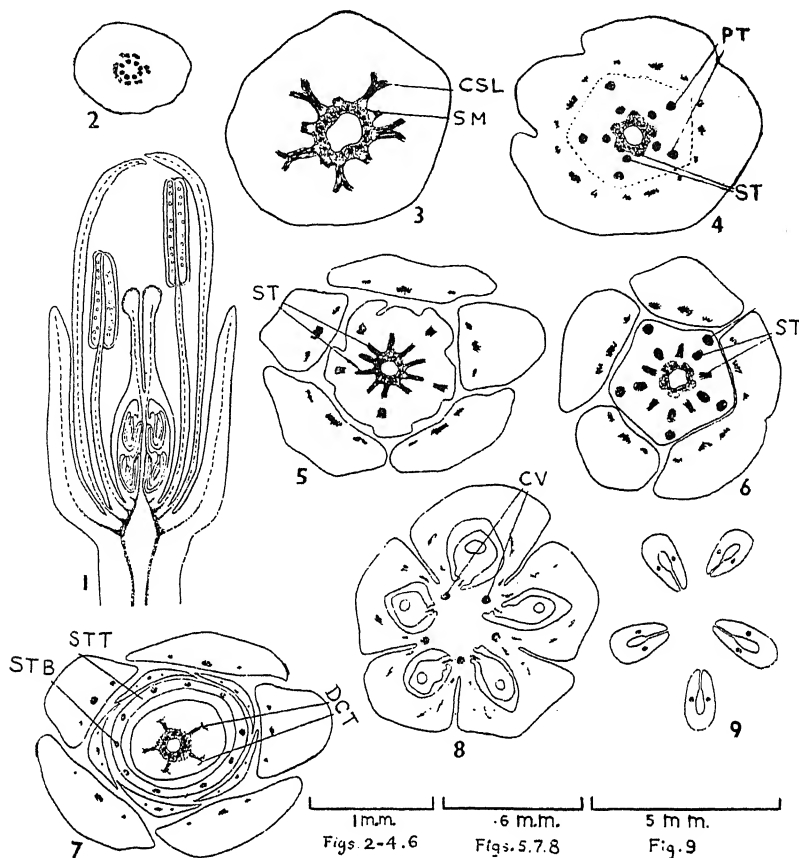
**Floral Anatomy.**—The pedicel shows a ring of discrete vascular bundles (Fig. 2). In *Sarcotheca glauca* there are groups of thick-walled cells outside the vascular bundles (Fig. 2). The common sepal lateral and sepal midrib traces arise in two close alternating whorls (Fig. 3). The former divide radially and demarcate the lateral traces of adjacent sepals. The sepal traces are followed by five petal traces (Fig. 4).

In *Sarcotheca glauca* the ten staminal traces arise in one whorl (Fig. 5) while in *S. oblongifolia* the androecium is obdiplostemonous, the antipetalous staminal traces arising earlier than the antisepalous staminal traces (Fig. 6).

After the separation of the staminal tube five dorsal carpellary traces arise (Fig. 7). These fade away at the base of loculi. The remaining stele organises into five common ventral bundles which lie along the septal radii (Fig. 8). Placentation is anatomically parietal. In the placental region ovular traces are given off from the common ventral bundles (Figs. 1, 8). They also give off branches into the ovary wall (Fig. 8). Towards the top of the ovary the common ventral bundles split into two each and these extend into the styles and finally end below the stigmas (Fig. 9).

**Discussion.**—The systematic position of *Sarcotheca* is controversial. Hallier (1921) placed the genus in the Oxalidaceae and this was followed later by Engler and Prantl (1931). Bentham and Hooker (1962–1883) included it in the Linaceae while Hutchinson (1959) created a separate family, Lepidobotryaceae, to include *Sarcotheca*, *Lepidobotrys* and *Dapania*. He placed the family under his Malpighiales.

*Sarcotheca* differs from *Lepidobotrys* and other Linaceae (Narayana, 1964; Narayana and Rao, 1966, 1969, 1971) and resembles Oxalidaceae in the absence of disc, obturator, in the presence of ephemeral dorsal carpellary traces and superposed ovules. Heimsch (1942) on the basis of wood anatomy arrived at the same conclusion and observed (p. 98) "It may be mentioned that on the basis of wood structure, the genus *Sarcotheca* justifiably is placed in the Oxalidaceae by Hallier (33, 34) rather than in the Linaceae". Thus, the results of



FIGS. 1-9. Figs. 1, 2, 5, 7, 8. *Sarcotheca glauca*. Figs. 3, 4, 6, 9. *Sarcotheca oblongifolia*. Fig. 1. Semidiagrammatic longitudinal section of flower showing the course of vascular bundles in the different floral parts. Figs. 2-9. Transverse sections of flower buds showing the origin distribution of the traces to the different floral parts. For explanation see text.

CSL, Common sepal lateral trace; SM, Sepal midrib trace; PT, Petal trace; ST, Staminal trace; STT, Staminal tube; STB, Staminal bundle; DCT Dorsal carpellary trace; CV, Common ventral bundle.

the present study and wood anatomy support the inclusion of *Sarcotheca* in the Oxalidaceae.

Our sincere thanks are due to Prof. Jafar Nizam for his interest and to Dr. K. Subramanyam, Director, Botanical Survey of India, for criticism and advice. We wish to express our deep sense of gratitude to Dr. W. A. Van Heel and the Director, Royal Botanic Gardens, Kew, for the materials.

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## REVIEWS AND NOTICES OF BOOKS

**"A Survey of Numerical Mathematics".** By David M. Young, and Robert T. Gregory. (Addison-Wesley Publishing Co., Reading, Mass., U.S.A.), 1973, in 2 volumes.

This book (in two volumes) gives a thorough and extensive survey of many algorithms for numerical computation. In addition to providing a working knowledge of practical techniques, there is a judicious mixture of mathematics and computational methods.

The highlights of the book are : Chapters 2 and 3 which deal with arithmetic and the errors in digital computers ; Chapter 9 which deals with the stability convergence and accuracy of solution of ordinary differential equations ; and Chapter 13 which deals with solution of linear systems using residue arithmetic. These materials have not been available to a working numerical analyst in one comprehensive book so far. The authors deserve credit for having been bold enough to include these rather unconventional but very useful chapters. The value of the book is greatly enhanced by the inclusion of these chapters.

Volume I, consists of eight chapters : Numerical analysis as a subject area ; Elementary operations with automatic digital computers ; Solution of equations ; Surveillance of Number ranges. Interpolation and approximation ; Numerical differentiation and quadrature ; and ordinary differential equations.

Volume II, has nine chapters : Ordinary differential equations—stability, convergence and accuracy ; Boundary value and eigenvalue problems ; Vectors, matrices and norms ; The solution of linear algebraic equations by direct methods ; Solving systems of linear algebraic equations using residue arithmetic ; The algebraic eigenvalue—eigenvector problem ; Partial differential equations—Elliptic boundary value problems ; Iterative methods for solving large linear systems ; Partial differential equations—Initial value problems.

A brief appendix of mathematical definitions and an excellent bibliography are given at the end.

Only one point that would affect the sale of this book is its very high price which would make it out of reach for most students and teachers. It is therefore necessary that every good library should obtain required number of copies of this book for ready reference. Computer Centres should also have this book readily available for users.

E. V. K.

**Progress in Plant Ecology in India (Vol. 1).** Edited by R. Misra, B. Gopal, K. P. Singh and J. S. Singh. (Today and Tomorrow's Printers and Publishers, New Delhi). September 1973. Price Rs. 40.00 or \$ 7.00.

The publication of this first of the projected two volume series reviewing the progress of plant ecology in India is a welcome indication of the fact that this discipline has now come of age in India. Although early botanists engaged in floristic studies did collect a great deal of incidental ecological information, genuine ecological studies in India may be said to have begun with Champion's survey of the forest types. Ecology has made steady progress since that event, and the present volume provides some excellent surveys of these accomplishments. Over half of the volume is taken up by Meher-Homji and K. C. Misra's review of phytogeography. These authors thoroughly cover the subject from both the historical or floristic and ecological or vegetational viewpoint. Unfortunately our knowledge of the geological history is so uncertain that much of the content of floristic phytogeography appears like building castles in the air. This is of course a shortcoming of all historical sciences, and the authors present a competent review of the basic data of the field. They are on much firmer ground when treating vegetational phytogeography and the review shows that we now have a rather satisfactory overall picture of the vegetational phytogeography of India although a great deal of detailed work still remains to be done. This discipline concerns itself with describing vegetation as a function of the climate. Now both vegetation and climate are very complex phenomena which require a large number of parameters for their adequate description. The rather primitive analytical tools of the phytogeographer limit his thinking to a space of three dimensions. He therefore uses all his ingenuity to reduce the description of the climate to just two parameters, sometimes with admirable results as is evident from Meher-Homji's work presented as Figs. 13, 14 and 15 of the review. It is nevertheless certain that an application of multivariate analysis techniques would greatly advance this field. As one of the leading authorities in this area C. R. Rao is an Indian, it is to be hoped that Indian plant ecologists will take a lead in this development. The second review in this book by P. S. Ramkrishnan deals with the genetic differentiation of the local popu-

lations in response to environmental pressures. This review brings home the remarkable conclusion that plant populations can become genetically differentiated with spatial isolation of no more than a few meters. The review chiefly concerns itself with mineral nutrients, an area to which Ramakrishnan himself has made important contributions. It is now known that analogous population differentiation can occur in response to biotic factors such as grazing, and in view of the economic significance of such factors it is to be hoped that future work on plant differentiation in India will embrace this dimension as well. The third review in the book is a summary of Navalkar's work on mangroves of Bombay area. He clearly establishes the role of exchangeable bases in the soil in favoring the different mangrove species. The last review of the ecology of arid and semi-arid zones in India is the only one to address itself to an applied problem. It is unfortunately somewhat of a disappointment in that the authors fail to provide a synthesis, but rather present what is tantamount to an annotated bibliography. There are also a large number of misprints, some serious as in "4th or 5th million B.C." instead of "4th or 5th millenium B.C." on page 128. Such misprints are however restricted to the fourth review, and the book on the whole is very well produced. I certainly recommend it whole heartedly to anybody interested in plant ecology. MADHAV GADGIL.

## ANNOUNCEMENTS

### Award of Research Degrees

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Zoology to Shri R. Ramachandra Rao, for his thesis entitled "Some Aspects of Enzyme Activity Pattern in Denervated Muscle", Ph.D. degree in Zoology to Shri S. Sanvar Basha for his thesis entitled "Some Aspects of Enzyme Systems and Protein Metabolism in Relation to Selected Exogenous protein and amino acid stress in sheep kidney cortex".

Berhampur University, has awarded the Ph.D. degree in Chemistry to Shri Rama Krushna Panda for his thesis entitled "Electron Transfer Reactions".

The M.S. University of Baroda, has awarded the Ph.D. degree in Botany to Shri Purshottam Deo-krishna Kulkarni for his thesis entitled "Primary Productivity of Periphyton and Macrophytes in Sayaji Sarovar at Baroda, India"; Ph.D. degree in Archaeology to Shri Swarnakamal Bhowmik for his thesis entitled "Early Copper and Bronze technology of Gujarat".

Karnatak University, Dharwar, has awarded the Ph.D. degree in Botany to Shri S. C. Hiremath, for his thesis entitled "Cytogenetical studies in Eleusine and its Allies"; Ph.D. degree in Zoology to Shri V. V. Thobbi for his thesis entitled "Studies on Some Systematic Insecticides".

Osmania University, Hyderabad, has awarded the Ph.D. degree in Astronomy to Shri K. Shankara Sastry for his thesis entitled "Changes in the Gravitational Energy of Galaxies due to Collisions"; Ph.D. degree in Chemistry to Shri Mohd. Khadir-uz-Zaman Khan for his thesis entitled "Hydrogena-tion of Selected Aromatic Compounds over Transi-tion Metal Catalysis"; Ph.D. degree in Geology to Shri Inkollu Sriman Narayana Murthy for his thesis entitled "Studies on Alkaline Rocks of Khammam District. A.P.".

### Books Received

*Ordinary Differential Equations: A First Course* (2nd Edition). By Fred Brauer, John A. Nohel. (Mrs. Frances R. McKenzie, Addison-Wesley Pub. Co., Inc., Reading, Mass., 01867), 1973. Pp. ix + 470. Price to be announced.

*Energy Metabolism*. By Eric G. Ball. (Addison-Wesley, W. A. Benjamin, Inc., Reading, Mass. 01867), 1973. Pp. xi + 84. Price: \$ 12.00; \$ 6.50.

*Basic Principles of Plasma Physics—A Statistical Approach*. By S. Ichimaru. (Addison-Wesley, W. A. Benjamin, Inc., Reading Mass. 01867), 1973. Pp. xviii + 324. Price: Cloth binding \$ 19.50; Paper binding, \$ 12.50.

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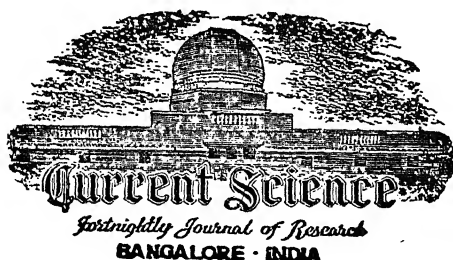
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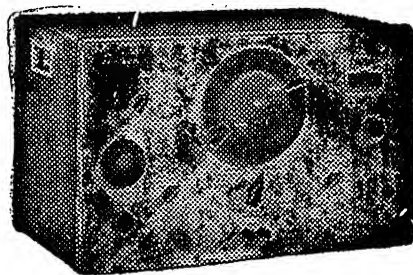
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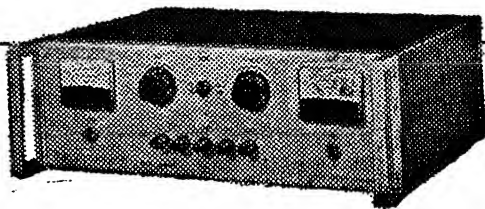
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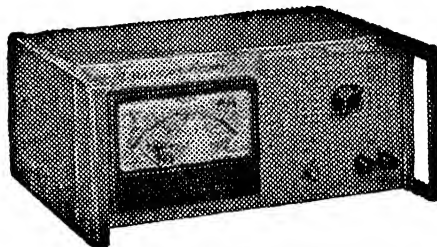
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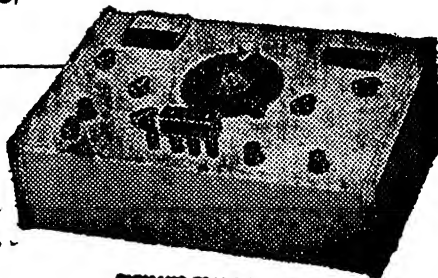
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# SHEATH EQUIVALENT CIRCUIT PARAMETERS BY PULSE MEASUREMENTS AND ITS USE FOR PLASMA DIAGNOSTICS

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It has been generally accepted<sup>1</sup> that the response of the sheath region, at the boundary between a foreign wall and a gas discharge plasma, to a probing r.f., signal can be described in terms of an equivalent circuit<sup>1,2</sup> consisting of a capacitance  $C_s$  in parallel with a resistance  $R_s$ . Several investigations<sup>1-6</sup> have been recently reported on the determination of  $C_s$  and  $R_s$ . The observed values of  $C_s$  and  $R_s$  have been utilised to obtain estimates for the plasma parameters<sup>1,2,6</sup>  $n_e$  (the charge carrier number density) and  $T_e$  (the electron temperature). The present paper reports observations on  $C_s$  and  $R_s$  by using a probe technique and a new procedure for the determination of  $n_e$  and  $T_e$  which yields fairly satisfactory estimates, in reasonably good agreement with those obtained from the single Langmuir probe data.

2. A d.c. maintained, gas discharge plasma in air was formed in a cylindrical discharge tube (radius = 1.3 cm) using electrodes of Al at a distance apart of 38.2 cm. The cathode was connected to the negative end of the H.T. whereas the anode was earthed through a resistance of 20 K $\Omega$ .

The sheath under investigation was formed at the surface of a large area<sup>1-3,6</sup> cylindrical Al probe (radius = 1.25 cm, height = 2.05 cm) positioned on the inner wall of the discharge tube, at a distance of 6.0 cm from the anode. The sheath parameters  $C_s$  and  $R_s$  were determined by injecting a rectangular voltage pulse<sup>5,7-9</sup>. (Pulse height = 5.4 V, Repetition frequency = 500 Hz, Pulse duration = 1200  $\mu$ S, rise time  $\leq 0.02$   $\mu$ S) at the anode end and observing the time constant of the current transient in the probe earth circuit on the C.R.O. The effective circuit for the pulse voltage has the form shown in Fig. 1 where  $R_1$  represents the known resistance across which the signal to be observed is obtained. If  $\alpha$  represents the ratio of the output to the input voltage and  $\tau$  the time constant of the circuit, it can be shown that

$$C_s = \frac{\tau}{(1 - \alpha) R_1}$$

and

$$R_s = \left( \frac{1 - \alpha}{\alpha} \right) R_1$$

The observables  $\alpha$  and  $\tau$  were both determined with the help of the C.R.O. (Tektronix 546). The observations for the floating condition of the probe

were taken when a suitable negative d.c. bias was applied, to make zero the d.c. current in the probe circuit. The floating potential  $V'_{CPF}$  (w.r.t. earth) was measured with the help of a high impedance d.c. V.T.V.M. Simultaneous determination of  $n_e$  and  $T_e$  were carried with the single Langmuir probe, for the sake of comparison. The experimental conditions selected were such that no oscillations or spatial non-uniformities were present in the plasma.

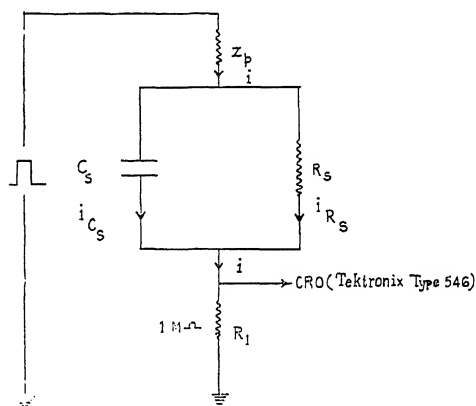


FIG. 1. Circuit for pulse measurement of equivalent circuit parameters  $C_s$  and  $R_s$  of the sheath.

Observations were taken for different discharge conditions which correspond to  $n_e$  values in the range (1-4)  $10^{15}$  m<sup>-3</sup> and  $T_e$  values (4-9)  $\times 10^4$   $^\circ$ K. Under these conditions  $\lambda_D/\lambda_e$  has values between 0.2 to 0.9 where  $\lambda_D$  represents the Debye shielding distance and  $\lambda_e$  the gas kinetic electron mean free path<sup>10</sup>. Representative values observed for  $C_s$  and  $R_s$  are shown in Table I. Figure 2 shows the variation of  $C_s$  with d.c. bias given to the probe. The values of the floating potential of the probe (w.r.t. anode determined from V.T.V.M. measurements) and of the plasma potential (w.r.t. anode from the Langmuir probe data) are indicated by a single and double arrows on these curves. It will be observed that the equivalent capacitance of the sheath has its minimum value when the probe potential approximately equals the plasma potential. Under these conditions one would expect, the sheath thickness to have a vanishingly small magnitude

but the space charge effects to become significant. The break point observed in the curve provides a fairly good method to locate the plasma potential.

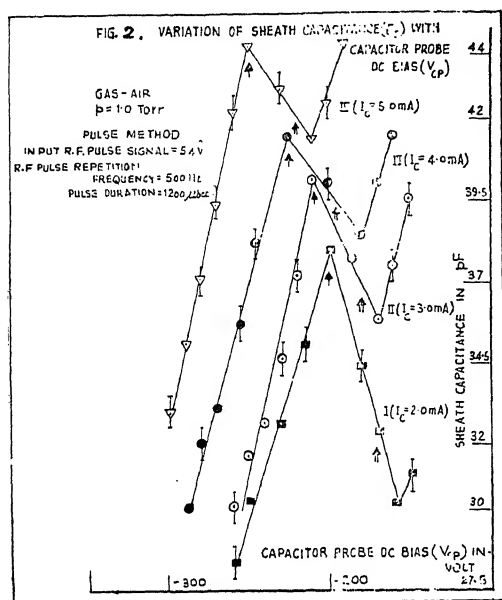


FIG. 2

TABLE I

Air discharge plasma;  $I = 2.0$  mA.

$p$ Torr	$C_{SF}$ $p^2$	$R_{SF}$ M $\Omega$	$\lambda_D$ in $10^{-3}$ m	$\frac{n_e}{(probe)}$ $\frac{n_e}{(pulse)}$	$\frac{T_e}{(probe)}$ $\frac{T_e}{(pulse)}$
	$\pm 4\%$	$\pm 6\%$			
0.20	20.4	0.32	68	1.4	0.7
0.35	25.6	0.26	55	1.2	0.7
0.60	30.6	0.22	46	1.4	0.8
1.00	37.4	0.21	37	1.2	0.8
1.40	43.1	0.19	31	1.1	0.9

In the present investigation,  $T_e$  has been determined from the usual expression for the floating potential<sup>10</sup> of the probe (w.r.t. plasma) by locating the plasma potential in this manner.

$$-V_{CPV} = \frac{kTe}{2e} \log e \left( \frac{T_e}{T_i} \cdot \frac{M}{m} \right) \quad (1)$$

where  $k$  = Boltzmann constant

$T_i$  = ion temperature  $\approx$  gas temperature

$M$  = mass of positive ion.

The electron number density has been calculated from the expression for the Debye shielding distance<sup>11</sup>

$$\lambda_D = \sqrt{\frac{\epsilon k T_e}{n_e e^2}} \quad (\text{MKS rationalised}) \quad (2)$$

on the assumption that when the probe is floating the sheath thickness<sup>11</sup>  $\lambda_D$  and that the sheath capacitance equals its geometrical value<sup>3</sup> given by

$$C_{SF} = \frac{2\pi\epsilon h}{\log_e \left( \frac{a}{a - \lambda_D} \right)} \quad (3)$$

where  $h$  = height of cylindrical probe

$a$  = its radius

$\epsilon$  = permittivity of sheath  $\approx \epsilon_0$ .

3. Typical results are given in Table I. The parameters as are represented in the non-dimensional form

$$\frac{n_e \text{ (Langmuir Probe data)}}{n_e \text{ (pulse measurements)}}$$

and

$$\frac{T_e \text{ (probe)}}{T_e \text{ (pulse)}}$$

The agreements between the two sets of values appears to be fairly good. The advantage of the present procedure is the reliance on the measurements of the equivalent capacitance alone which involves less error and is less likely to be influenced by surface contamination of the capacitor probe.

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# GROUP ELECTRONEGATIVITIES OF A FEW RING RADICALS FROM MICROWAVE SPECTROSCOPY

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THE group electronegativity of a radical  $X_nC$  derived from the molecule  $X_nC-A$  (the radical  $X_nC$  always bonded through C) can be regarded as the electronegativity of an atom C perturbed by its chemical bonding to A. Group electronegativities of the group have been estimated from various methods like Infra red, solubility, basicity and coupling potential and NMR data. Hinze *et al.*<sup>1</sup> and Gordy<sup>2</sup> have developed methods of calculating group electronegativities.

Hinze and co-workers<sup>1</sup> introduced the idea of orbital electronegativity which is defined as the derivative of the energy of the atom with respect to the charge in the orbital, *i.e.*, the number of electrons in that orbital ( $0 \leq n_j \leq 2$ ). They have expressed the energy  $E$  of the atom

$$E(n_j) = a + bn_j + cn_j^2$$

where  $a$ ,  $b$  and  $c$  are constants and  $n_j$  is the occupation number of the  $j^{\text{th}}$  orbital. Then the  $j^{\text{th}}$  orbital electronegativity is

$$\chi_j = \frac{\partial E}{\partial n_j} = b + 2cn_j \quad (1)$$

Gordy's<sup>2</sup> approach, subsequently modified by Wilmshurst<sup>3</sup> consists in relating the electronegativity of an atom with the energy of a valence-state electron arising from its interaction with the unscreened nucleus at a distance corresponding to covalent-bond formation. Hence for a radical  $x_nC\cdot$ , the electronegativity can be written as

$$\chi = 0.31 \frac{n^* + 1}{r} + 0.50 \quad (2)$$

where  $r$  is the covalent radius of atom C.  $n^*$ , a modified valence electron number, can be considered to be composed of three terms: (i) non-bonded electrons, (ii) bonded electrons and (iii) resonance electrons and can be written as

$$n^* = (n - p) + \frac{2m\chi_c}{(\chi_c + \chi_x)} + \frac{s\chi_c}{(\chi_c + \chi_x)}$$

where  $n$  is the number of valence electrons on the free atom C,  $p$  is the number of electrons taking part in the bonding to A,  $m$  the number of two electron bonds between C and A and  $s$  the number of resonance contributors.

Different relations relating ionic character to the electronegativity differences have been postulated.

However, Gordy's<sup>2</sup> relation that ionic character  $i$  for a bond A-B

$$i = \frac{(\chi_A - \chi_B)^2}{2} \quad (3)$$

seems to be most generally followed.  $\chi_A$  and  $\chi_B$  refer to the electronegativities of atoms A and B respectively. From the theory of nuclear quadrupole coupling constants, it is possible to estimate the ionic character in a bond and it is given by

$$i = \left(1 - s + d - \frac{\pi}{2}\right) - U_p \quad (4)$$

where the amount of  $s$  and  $d$  hybridization are indicated by  $s$  and  $d$  and  $\pi$  refers to the double bond character and

$$U_p = - \frac{(eQq)_{mol}}{(eQq)_{at}}$$

represents the number of unbalanced  $p$  electrons.  $(eQq)_{mol}$  is the quadrupole coupling constant of the atom in a molecule determined by microwave spectroscopy and  $(eQq)_{at}$  that of the free atom. In most cases  $d$  character is negligible and hence can be ignored. Regarding the contribution due to hybridization, Gordy<sup>4</sup> prefers to put  $s$  always equal to zero, while Townes and Dailey<sup>5</sup> have assigned a value of 0.15 for  $s$  if the electronegativity of the halogen is 0.25 units more than that of its partner. The  $\pi$ -character in the bond is given by

$$\pi = \frac{2}{3} \frac{(\chi_{xx} - \chi_{yy})}{(eQq)_{at}} \quad (5)$$

when  $\chi_{xx}$  and  $\chi_{yy}$  are the components of the quadrupole constant along the  $x$  and  $y$  axes respectively. Using Gordy's approach, the eqn. (4) can then be written as<sup>4</sup>

$$i = 1 - \frac{\pi}{2} + \frac{(eQq)_{mol}}{(eQq)_{at}} \quad (6)$$

Recently a number of halogen substituted benzene, pyridine and thiophene compounds have been studied by microwave spectroscopy. Hence if the halogen is considered as atom A and the group to be B, then combining eqns. (3) and (6) we get

$$\chi_B = \chi_{mol} - 2 \left[ 1 - \frac{\pi}{2} + \frac{(eQq)_{mol}}{(eQq)_{at}} \right] \quad (7)$$

Using the quantities listed in Table I, the electronegativities of various groups have been evaluated from the quadrupole coupling constants determined from microwave spectroscopy. These are given in

Table II through V. However, if we include the  $s$  character as per the prescription of Townes and Dailey<sup>5</sup> the group electronegativity increases by 10% of its quoted value.

TABLE I

Quadrupole coupling constants and electronegativity of halogens<sup>4</sup>

Atom	$(eQq)_{at}$ (MHz)	$\chi_{hal}$
<sup>35</sup> Cl	109.74	3.0
<sup>37</sup> Cl	86.51	3.0
<sup>79</sup> Br	-769.76	2.8
<sup>81</sup> Br	-643.03	2.8
I	2292.71	2.55

TABLE II

Group electronegativity of Phenyl radical

Molecule	$(eQq)_{mol}$ (MHz)	$\pi$	$\chi_g$	$(\chi_g)_{av}$	$\chi_g$ from other methods <sup>8</sup>	Ref.
Chlorobenzene- <sup>35</sup> Cl	-71.09	0.0326	2.36			6
Bromobenzene- <sup>79</sup> Br	558.9	0.0226	2.30			6
				2.30	3.13	
Bromobenzene- <sup>81</sup> Br	464.1	0.0221	2.27			6
Iodobenzene	-1892.1	0.0175	2.27			7

TABLE III

Group electronegativity of Pyridyl radical

Molecule	$(eQq)_{mol}$ (MHz)	$\pi$	$\chi_g$	$(\chi_g)_{av}$	Ref.
2 Chloropyridine- <sup>35</sup> Cl	-74.29	0.045	2.44		9-10
„ <sup>37</sup> Cl	-58.24	0.044	2.43		1 1 9
3 Chloropyridine- <sup>35</sup> Cl	-68.77	0.035	2.36		11,12
				2.34	
2 Bromopyridine- <sup>79</sup> Br	552.2	0.049	2.28		13
„ <sup>81</sup> Br	460.4	0.046	2.28		13
4 Bromopyridine- <sup>79</sup> Br	557.5	0.0377	2.24		13
„ <sup>81</sup> Br	475.4	0.0339	2.31		13

TABLE IV

Group electronegativity of monofluorophenyl radical

Molecule	$(eQq)_{mol}$ (MHz)	$\pi$	$\chi_g$	Ref.
$m$ fluorochlorobenzene- <sup>35</sup> Cl	-76.00	0.044	2.43	14

TABLE V

Group electronegativity of Thiophene radical

Molecule	$(eQq)_{mol}$ (MHz)	$\pi$	$\chi_g$	$(\chi_g)_{av}$	Ref.
2 Chlorothiophene- <sup>35</sup> Cl	-76.15	0.011	2.40		15
	-75.54	0.010			
				2.40	
„ <sup>37</sup> Cl	-66.07	0.008	2.40		
	-59.59				

It may be noted from Tables I and II that the group electronegativity decreases as the bonding of the radical changes from chlorine to iodine. Because these are conjugated systems, the uncertainty in the evaluation of the group electronegativity is likely to be large. Nevertheless it is interesting to note that the group electronegativities for these widely varying radicals are very nearly equal to 2.35.

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## AMINO ACID COMPOSITION OF SOME WILD LEGUMES

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### ABSTRACT

Twenty-eight non-edible wild leguminous seeds were analysed for their proximate composition and free amino acid pattern. Distribution of total nitrogen in the various fractions of protein isolates were studied. Essential amino acids in the seed meal hydrolysates were assayed.

Tryptophan, cystine and methionine invariably appear to be the most common limiting amino acids in most of the seeds. No single seed has been found to be nutritionally complete with respect to essential amino acid content.

**S**EVERAL novel sources of proteins that have hitherto not been employed in human nutrition are now being investigated<sup>1</sup> in view of increasing demands of proteins by a growing world population. For example, oil seeds such as pea nut, cotton seed, sesame, soy bean, sunflower seed, and coconuts, etc., have been employed as sources of proteins. All-vegetable mixtures rich in protein content are prepared commercially and sold at low cost<sup>2</sup>. Other unconventional protein sources that are presently being developed and nutritionally evaluated include various algae like chlorella and blue alga, seafoods like weeds and planktons, yeasts and micro-organisms. Nevertheless, apart from their nutritional efficacy, cost of production and availability, the question of their acceptance by the consumers is an important factor in recommending them for human use. Some of these novel proteins for instance, possess unpleasant odour, acrid taste and unattractive colour.

Dry legumes have occupied an important position in human dietary since agriculture began and man adopted a settled life<sup>3</sup>. Leguminous plants belong to the second largest family of seed plants consisting of about 600 genera with 13,000 species. Of these however, only some twenty species have been considered suitable for human consumption and have hitherto been investigated by nutritionists while the rest have grossly been discarded and branded as "wild and non-edible" till recently<sup>4,5</sup>. The presence of cyanogenetic factors<sup>6</sup>, hemagglutinins and toxic substances that produce lathyrism are some of the reasons for such downright disregard for these protein-rich sources in an age of severe protein scarcity and malnutrition.

Many of these wild leguminous plants grow most abundantly in tropical countries that are also the chief centres of protein undernutrition. They survive even under inhospitable and acute adverse ecological conditions and yield annually large quantities of seed-bearing pods of all sizes upto

a meter in length with thick, fat and protein-rich cotyledons.

Therefore, with a view to explore the possibility of their inclusion in animal dietary a number of wild leguminous seeds were collected, botanically identified and investigated chemically to explore their nutritional efficiency.

### MATERIALS AND METHODS

Wild leguminous seeds of *Acacia arabica*, *Acacia catechu*, *Acacia melanoxylon*, *Acacia suma*, *Albizia lebbek*, *Albizia moluccana*, *Albizia odoratissima*, *Albizia richardiana*, *Bauhinia alba*, *Bauhinia acuminata*, *Bauhinia macrostachya*, *Bauhinia malabarica*, *Bauhinia monandra*, *Bauhinia variegata*, *Cassia absus*, *Cassia grandis*, *Cassia marginata*, *Cassia obtusifolia*, *Cassia occidentalis*, *Cassia renigera*, *Cassia siamea*, *Crotalaria juncea*, *Crotalaria medicaginea*, *Dolichos biflorus*, *Erythrina indica*, *Glycine hispida*, *Mucuna pruriens* and *Pithecellobium dulce* were collected and botanically identified. They were powdered to 100-mesh in a hand grinder and stored in air-tight bottles. Moisture, ash, minerals, ether extractives and crude proteins were determined as described previously<sup>7,8</sup>. Niacin was assayed by the method of Swaminathan<sup>9</sup> and ascorbic acid, according to Roe and Kuether as described in Practical Physiological Chemistry by Hawk, Osier and Summerson<sup>10</sup>.

Nitrogen-free extract percentage was calculated by subtracting the total of the percentages of crude protein, ether extractives, crude fibre and ash on moisture-free basis from 100. This presumably constitutes the total carbohydrate percentage.

Seed powders were defatted with petroleum ether (B.P. 60–80°C) in a Soxhlet extractor.

*Extraction and isolation of free amino acids* from seed powders were made by stirring the defatted seed powder (1.0 gm) with warm ethanol (10 ml, 70%, v/v) for 30 minutes. After centrifugation the residue was re-extracted with ethanol,

centrifuged and the two supernatants combined. This process was repeated (8–9 times) till the supernatant was negative to ninhydrin test. The pooled supernatant was then evaporated to dryness *in vacuo*, dissolved in distilled water (0.5–1.0 ml), centrifuged and the clear supernatant (2–10  $\mu$ l) was employed for qualitative free amino acid analysis by paper partition chromatography on Whatman No. 1. filter-paper sheets.

Free amino acids were detected by the two-dimensional technique of Datta, Dent and Harris<sup>11</sup> employing phenol (80%, w/v)-NH<sub>3</sub> and butan-1-ol-acetic acid-water (4 : 1 : 5) as developing solvents. The chromatograms after development were sprayed with ninhydrin (0.1%, w/v) in butan-1-ol. The identity of the various amino acids was confirmed by using specific spray reagents<sup>12</sup>.

*Preparation of protein hydrolysates from seed meals for amino acid analysis:* 0.10 gm of the dry residue obtained after the isolation of free amino acids was hydrolyzed with hydrochloric acid (7 ml, 6 N) in an evacuated sealed tube by heating it in an air oven maintained at a temperature of 100–110° C for about 20–22 hours till the hydrolysate was negative to burette test. Under the conditions employed, complete hydrolysis of the proteins to amino acids was achieved. The acidic hydrolysate was made acid-free to pH 4–5 by repeated distillation *in vacuo*. The residue dissolved in a known volume of water, was employed both for qualitative and quantitative amino acid estimations.

Presence of amino acids in the seed protein hydrolysates was detected as described above. Tryptophan was tested and identified in the alkaline hydrolysate prepared by refluxing another sample of free amino acid-free seed powder with sodium hydroxide (10 ml, 5 N) till negative to the burette test.

*Quantitative estimations of amino acids* in seed protein hydrolysates were made by the elution method of Price<sup>13</sup>. The chromatograms spotted with the seed meal protein hydrolysates and developed by the two-dimensional technique, were dipped in ninhydrin solution (0.5%, w/v) in acetone-acetic acid mixture (1%, v/v) and heated at 90° C for 30 minutes. The amino acid spots were identified by comparison with chromatograms of known reference amino acids run simultaneously under identical conditions.

The coloured amino acid spots of identified amino acids were cut individually into small pieces taking care not to contaminate them and eluted with aqueous ethanol (7 ml, 60%, v/v) in centrifuge tubes. The tubes were well-agitated with a glass rod and centrifuged at 1500 r.p.m. for about

10 min. and the optical density of the supernatants were measured at 750 m $\mu$  against a reagent blank prepared from a paper treated in an identical manner. All estimations were made in triplicates. The percentage error by this method was  $\pm 8$ .

Proline was measured at 440 m $\mu$ . Amino acids were calculated from a calibration curve prepared earlier from standard glycine solutions of known concentration.

Tryptophan was assayed in the alkaline hydrolysate by employing the method of Inglis and Leaver<sup>14</sup>.

*For the fractionation and isolation of proteins from defatted seed meals* Mitchell's<sup>15</sup> solubility method as detailed in an earlier communication<sup>16</sup> was adopted. The isolated globulins and albumins were purified by the method of Esh and De<sup>17</sup>. Electrophoresis of the purified proteins was performed on an LKB 3276 unit employing Carl Schleicher and Schull No. 2043 13 (120 g/m<sup>2</sup>) filter-paper strips using several buffer solutions (acetate buffer pH 5.0, citrate buffer pH 6.0 and phosphate buffer pH 8.0).

## RESULTS AND DISCUSSION

Table I reveals that barring a few varieties of Cassia quite a number of wild inedible leguminous seeds could be good sources of proteins.

Qualitative amino acid analyses in the seed powders showed the presence of 9–18 amino acids in the free state. They were  $\alpha$ -alanine,  $\alpha$ -amino-butyric acid, arginine, aspartic acid, citrulline, cysteic acid, cystine, glutamic acid, glutamine, glycine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, proline, hydroxy-proline, serine, taurine, threonine, tryptophan, tyrosine and valine. Each seed appears to have its own pattern although most of them in general, showed the absence of the essential amino acids phenylalanine, tryptophan and the sulphur containing methionine as well as cystine and cysteic acid. Some of them also revealed the presence of some unidentified ninhydrin positive substances.

Qualitative amino acid analyses of the seed protein hydrolysates revealed them to possess more or less similar amino acid patterns consisting of 15–19 usually occurring amino acids. Among the essential amino acids methionine and tryptophan singly or jointly appeared to be the common limiting factors in general. In addition, the other sulphur containing amino acid cystine also was observed to be present only in few seeds.

Unlike the green leafy vegetables which are well balanced with respect to all essential amino acids except methionine, practically all the legume proteins investigated lack mainly in sulphur containing

TABLE I

Proximate chemical composition of some nonedible leguminous seeds  
(Results expressed as percentage on dry weight basis)

SEED	Crude Protein	Ether Extractives	Nitrogen free Extract	Crude fibre	Total ash	Calcium mg/100 g	Phosphorus mg/100 g	Iron mg/100 g	Niacin mg/100 g	Ascorbic acid mg/100 g
<i>Acacia arabica</i>	26.4	3.3	62.9	2.7	4.7	673	426	4.95	3.17	4.51
<i>Acacia catechu</i>	44.2	6.6	41.6	3.5	4.1	578	445	5.15	1.63	6.11
<i>Acacia melanoxylon</i>	39.7	5.5	47.0	2.9	4.9	707	412	7.19	2.93	6.98
<i>Acacia suma</i>	31.9	5.5	54.0	3.7	4.9	718	396	7.10	2.45	6.93
<i>Albizia lebbek</i>	39.5	6.8	45.3	4.2	4.2	172	620	11.50	5.21	1.75
<i>Albizia moluccana</i>	33.5	5.6	52.5	3.8	4.6	732	365	4.90	1.46	6.58
<i>Albizia odoratissima</i>	38.6	6.7	47.2	2.9	4.6	682	338	3.81	1.06	2.83
<i>Albizia richardiana</i>	40.0	7.4	41.3	3.5	3.8	189	570	12.80	4.62	2.68
<i>Bauhinia alba</i>	30.7	15.4	46.6	3.2	4.1	273	192	3.91	2.84	1.62
<i>Bauhinia accuminata</i>	31.8	18.2	43.7	2.9	3.4	285	165	3.20	4.13	1.08
<i>Bauhinia macrostachya</i>	22.8	20.7	50.9	2.1	3.5	105	267	8.10	12.82	4.26
<i>Bauhinia malabarica</i>	27.0	17.8	48.6	3.2	3.4	142	299	13.30	9.20	6.84
<i>Bauhinia monandra</i>	31.0	22.7	39.5	3.7	3.1	121	201	14.40	15.81	4.28
<i>Bauhinia variegata</i>	34.1	16.5	43.1	3.2	3.1	312	120	4.29	3.91	1.00
<i>Cassia absus</i>	36.8	6.8	49.4	2.6	4.4	135	680	22.40	3.21	2.56
<i>Cassia grandis</i>	12.8	5.0	75.6	3.7	2.9	124	203	8.10	6.59	2.88
<i>Cassia marginata</i>	16.5	6.8	68.4	4.6	3.7	149	191	11.36	9.15	6.28
<i>Cassia obtusifolia</i>	23.9	5.2	60.9	4.1	5.2	153	215	10.57	8.38	3.56
<i>Cassia occidentalis</i>	35.2	5.0	51.3	3.8	4.7	160	226	10.98	7.59	4.29
<i>Cassia renigera</i>	12.9	3.5	75.8	4.5	3.3	133	178	12.67	9.03	5.42
<i>Cassia siamea</i>	21.8	6.9	62.6	5.3	3.4	155	430	8.20	3.82	3.10
<i>Crotalaria juncea</i>	32.4	2.8	59.6	2.1	3.1	201	326	7.91	2.95	1.39
<i>Crotalaria medicaginea</i>	47.5	3.4	43.2	2.6	3.7	167	352	8.57	3.15	2.05
<i>Dolichos biflorus</i> (Edible)	21.3	2.3	69.6	2.9	3.9	269	360	9.20	2.07	0.65
<i>Erythrina indica</i>	23.1	15.7	53.1	3.5	4.6	214	201	12.27	1.28	1.97
<i>Glycine hispida</i> (Edible)	46.8	21.1	23.6	3.2	5.3	317	272	10.79	3.14	1.17
<i>Mucuna pruriens</i>	29.3	9.0	53.7	4.1	3.9	238	159	13.52	3.64	4.78
<i>Pithecellobium dulce</i>	24.4	11.7	57.2	3.7	3.0	254	570	3.55	4.87	1.62

amino acids—methionine and cystine as well as in tryptophan. However, many of them appear to be fairly good sources of lysine, threonine and leucine-isoleucine and other essential amino acids. Nevertheless, in the total absence of one or two essential amino acids these proteins cannot possibly be expected to possess nutritive value.

Table III shows that successive extraction of seed meals with water and sodium chloride, solubilizes 41–86% of the total nitrogen consisting of albumin,

globulin and non-protein nitrogen. Globulin forms the major fraction of total nitrogen. Prolamin constitutes a small fraction (1–5%), non-protein nitrogen accounts for 4–18% while 1–6% remains unextracted in the residue.

Electrophoresis of purified globulin fractions invariably afforded mainly one band. The present findings are in conformity with the previous reports<sup>17,18</sup> on the nature of seed proteins isolated in the manner described in the present investigation.



TABLE II

Essential amino acid content of some nonedible leguminous seeds  
(Results expressed as g amino acid/16 g N)

SEED	His.	Lys.	Met.	Cys.	Phe.	Tyr.	Phe. + Tyr.	Leu + Ile	Val.	Thr.	— + T.p.*	Total
<i>Acacia arabica</i>	3.7	4.3	0.4	0.49	3.6	1.58	5.18	8.8	4.2	3.3	+	30.37
<i>Acacia catechu</i>	2.3	4.2	0.5	..	3.8	1.2	5.08	9.4	3.0	2.8	+	27.28
<i>Acacia melanoxydon</i>	2.1	3.8	+	0.70	1.6	1.98	3.59	7.3	3.8	2.1	—	23.38
<i>Acacia suma</i>	2.9	2.9	2.0	—	3.8	1.39	5.19	6.8	2.5	1.9	0.4	24.59
<i>Albizia lebbek</i>	2.8	5.8	1.3	—	3.7	0.90	4.60	5.6	2.6	1.8	+	27.70
<i>Albizia moluccana</i>	2.3	6.2	0.9	—	5.6	0.78	6.38	7.2	1.8	2.4	—	27.18
<i>Albizia odoratissima</i>	2.2	5.2	1.2	—	5.0	0.70	5.70	7.2	1.6	1.5	1.4	26.00
<i>Albizia richardiana</i>	3.8	7.8	0.8	—	4.0	0.49	4.49	8.9	3.3	3.3	+	32.39
<i>Bauhinia accuminata</i>	1.7	5.8	0.3	0.27	4.3	1.64	5.94	6.9	6.2	4.3	1.1	32.51
<i>Bauhinia alba</i>	1.2	4.9	0.5	0.30	3.3	1.65	4.95	7.9	4.2	2.3	+	26.25
<i>Bauhinia macrostachya</i>	1.6	4.7	0.4	—	1.5	0.77	2.27	5.6	3.4	1.6	+	19.57
<i>Bauhinia malabarica</i>	1.9	3.6	+	—	2.0	0.33	2.33	4.2	3.5	2.9	—	18.43
<i>Bauhinia monandra</i>	2.2	4.7	+	—	1.4	0.84	2.34	7.7	4.8	2.2	—	23.94
<i>Bauhinia variegata</i>	2.3	4.8	0.4	0.29	3.4	1.75	5.15	6.2	6.0	4.2	—	29.34
<i>Cassia absus</i>	1.5	3.1	1.2	—	3.2	0.81	4.01	4.6	5.6	4.5	—	24.51
<i>Cassia grandis</i>	1.5	3.9	1.0	—	1.4	0.48	1.88	6.9	2.5	2.8	1.1	21.58
<i>Cassia marginata</i>	1.1	4.3	1.5	—	2.8	0.38	3.18	8.9	2.4	2.0	—	23.38
<i>Cassia obtusifolia</i>	2.8	6.5	0.6	—	2.8	1.63	4.43	7.1	2.3	2.9	1.6	28.23
<i>Cassia occidentalis</i>	1.2	7.5	0.5	—	2.2	1.25	3.45	6.8	3.7	3.8	1.2	28.15
<i>Cassia renigera</i>	2.5	4.4	1.1	—	3.7	0.68	4.38	6.1	3.6	1.3	0.7	24.08
<i>Cassia siamea</i>	3.1	4.4	1.3	—	1.1	0.23	1.33	6.4	3.5	3.7	1.2	24.93
<i>Dolichos biflorus</i>	3.1	3.8	0.3	0.79	3.4	1.13	4.53	2.4	4.6	2.8	1.1	23.24
<i>Glycine hispida</i>	2.7	3.9	0.4	0.83	3.9	1.44	5.34	7.2	5.6	3.3	1.4	30.67
<i>Mucuna pruriens</i>	2.8	1.7	0.3	—	1.3	0.63	1.93	7.5	2.6	1.3	1.3	19.43
<i>Pithecellobium dulce</i>	2.6	4.0	0.2	1.13	4.1	2.07	6.17	5.6	4.7	2.4	1.8	28.60

+ Present

— Absent

\* Estimated in alkaline hydrolysates of seed meals.

TABLE III  
Distribution of nitrogen in some nonedible leguminous seed proteins  
(Expressed as per cent of total N)

SEED	Water-soluble (Alb + Glob + NPN)	Albumin	Globulin (A)	Non-protein nitrogen (NPN)	5% NaCl soluble (Globulin B)	Total Globulin (A+B)	75% Ethanol soluble (Pro-lamin)	0.25% NaOH soluble (Glutelin)	Residue
<i>Acacia arabica</i>	41.3	7.1	21.1	13.1	25.3	46.4	1.2	21.1	3.1
<i>Acacia catechu</i>	46.9	6.2	26.3	14.4	26.0	52.3	3.2	16.4	7.5
<i>Acacia melanoxylon</i>	44.8	5.9	20.5	18.4	22.6	43.1	3.6	24.7	4.3
<i>Acacia suma</i>	40.8	4.1	24.7	12.0	25.8	50.5	4.6	20.3	8.5
<i>Albizzia lebbek</i>	78.4	13.9	47.9	14.8	8.8	58.5	0.9	4.3	7.6
<i>Albizzia moluccana</i>	50.4	6.8	30.0	13.6	28.6	58.6	2.3	12.6	6.1
<i>Albizzia odoratissima</i>	49.1	5.7	29.3	14.1	27.8	57.1	2.7	15.2	5.2
<i>Albizzia richardiana</i>	85.9	17.5	53.2	15.3	2.9	56.1	0.4	2.4	8.4
<i>Bauhinia accuminata</i>	60.5	8.6	46.5	5.4	18.6	65.1	1.4	12.2	7.3
<i>Bauhinia alba</i>	67.4	13.8	47.6	6.1	22.4	70.0	3.2	2.7	4.2
<i>Bauhinia macrostachya</i>	73.2	7.2	61.0	5.0	9.3	70.3	2.4	9.1	6.0
<i>Bauhinia malabarica</i>	59.9	6.7	39.8	13.4	20.9	60.7	3.1	7.3	8.8
<i>Bauhinia monandra</i>	49.2	5.1	39.7	4.3	17.0	56.7	3.5	20.1	10.2
<i>Bauhinia variegata</i>	72.7	4.6	60.0	8.1	12.5	72.5	2.1	8.6	4.1
<i>Cassia absus</i>	46.0	8.7	33.0	4.3	18.8	51.8	0.7	28.4	6.1
<i>Cassia grandis</i>	46.1	10.3	28.0	8.0	16.4	44.4	2.5	32.3	8.5
<i>Cassia marginata</i>	47.0	15.3	26.4	5.3	18.8	45.2	1.2	28.4	4.5
<i>Cassia obtusifolia</i>	47.8	9.5	32.5	5.8	13.2	45.7	0.8	29.4	8.8
<i>Cassia occidentalis</i>	52.5	12.5	33.8	6.2	12.7	46.5	0.9	25.4	8.5
<i>Cassia renigera</i>	51.8	14.8	32.1	4.9	15.4	47.5	1.6	25.0	6.2
<i>Cassia siamea</i>	49.1	12.6	27.6	9.7	17.6	45.2	4.7	23.0	5.6
<i>Dolichos biflorus</i>	69.9	15.4	49.8	4.1	13.2	63.0	2.0	6.8	8.1
<i>Glycine hispida</i>	71.0	3.5	61.5	6.0	6.2	67.7	1.0	6.0	15.7
<i>Leucina glauca</i>	58.6	8.6	35.8	14.2	29.1	64.9	1.8	30.1	3.0
<i>Mucuna pruriens</i>	79.5	5.6	66.9	6.9	16.3	83.2	Traces	2.8	1.4
<i>Pithecellobium dulce</i>	70.5	7.4	58.3	4.8	9.2	67.5	1.3	5.2	5.9
<i>Phaseolus aconitifolius</i>	52.3	5.1	33.2	13.6	23.6	56.8	3.8	12.3	7.2

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## LETTERS TO THE EDITOR

MOLECULAR VIBRATIONS IN ISOTOPIC  
MOLECULES—HOF AND DOF

RECENTLY, Appelman *et al.*<sup>1</sup>, for the first time reported the infrared spectra of the two elusive molecules HOF and DOF in the gas phase. The present communication reports the normal co-ordinate analysis of these molecules using General Valence Force Field and Urey-Bradley Force Field. G-F matrix elements and symmetry co-ordinates are the same as those used by Venkateshwarlu *et al.*<sup>2</sup>.

From Table I, it is evident that the stretching force constants  $f_d$ ,  $f_r$  and  $K_D$ ,  $K_R$  in GVFF and

UBFF respectively increase, on isotopic substitution of the Hydrogen atom by Deuterium whereas the bending force constants  $f_a$  and  $H_a$ , decrease. The values obtained in the two cases are in agreement.

The mean square amplitude quantities (Table II) and mean amplitudes of vibration (Table III) have been evaluated at three temperatures using Cyvin's method<sup>3</sup>. It is observed that the bonded mean amplitude decreases with the replacement of Hydrogen atom by Deuterium atom but the other bonded amplitude is affected slightly. The non-bonded mean amplitude also diminishes. Mean amplitudes

TABLE I

GVFF and UBFF constants (in m dynes/Å) of some bent XYZ molecules

HOF		DOF	
GVFF	UBFF	GVFF	UBFF
$f_d = 7.154$	$K_D = 7.169$	$f_d = 7.380$	$K_D = 7.404$
$f_r = 4.430$	$K_R = 4.445$	$f_r = 4.609$	$K_R = 4.632$
$f_{dr} = 0.053$	$F = -0.019$	$f_{dr} = 0.072$	$F = -0.029$
$f_{da} = -0.005$	$F' = -0.002$	$f_{da} = -0.005$	$F' = -0.003$
$f_{ra} = -0.163$		$f_{ra} = -0.215$	
$f_a = 0.536$	$H_a = 0.544$	$f_a = 0.505$	$H_a = 0.517$

TABLE II

Mean square amplitudes of vibration (in  $10^{-4} \times \text{Å}$ ) for some XYZ bent molecules

Symbol	HOF			DOF		
	T = 0° K	T = 298° K	T = 500° K	T = 0° K	T = 298° K	T = 500° K
$\sigma_{r1}$	.. 49.702	49.702	49.702	35.566	35.566	35.566
$\sigma_{r2}$	.. 14.309	14.414	15.103	21.256	22.032	23.365
$\sigma_a$	.. 231.949	238.739	276.658	126.427	129.135	147.372
$\sigma_{r1r2}$	.. -0.713	-0.713	-0.713	-0.964	-0.964	-0.964
$\sigma_{r1a}$	.. -2.976	-2.976	-2.976	-4.029	-4.029	-4.029
$\sigma_{r2a}$	.. -5.266	-5.274	-5.356	-6.932	-7.043	-8.864
$\sigma_t$	.. 207.014	216.322	246.544	133.899	137.582	141.863

TABLE III  
Mean amplitude of vibration (in  $10^{-2} \times \text{\AA}$ ) of some bent XYZ molecules

Symbol		HOF			DOF		
		T = 0° K	T = 298° K	T = 500° K	T = 0° K	T = 298° K	T = 500° K
$U_{x-y}$	..	7.049	7.049	7.049	5.964	5.964	5.964
$U_{x-z}$	..	3.783	3.796	3.882	4.610	4.694	4.834
$U_{y---z}$	..	14.387	14.707	15.701	12.774	12.791	12.810

for both bonded and non-bonded distances increases with the increase in the temperature.

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### NMR STUDIES ON POLY (S-BENZYL-L-CYSTEINE) IN SOLUTION

IN a recent review by one of us<sup>1</sup> the need for a systematic study of the  $\beta$ -structure in polypeptides by various physical techniques was emphasized. As part of our studies in this direction, we report in this communication our results on the nuclear magnetic resonance (NMR) studies on poly (S-benzyl-L-cysteine) [PSBC] in mixtures of deuterated chloroform ( $\text{CDCl}_3$ ) and dichloroacetic acid (DCA). The results indicate that a conformational change occurs in the polypeptide in going from  $\text{CDCl}_3$  to DCA solution; this change is likely to be a  $\beta$ -structure  $\rightarrow$  coil transition.

PSBC was obtained from Sigma Chemical Co., U.S.A. (M.wt.  $\approx 5000$ ). DCA from Riedel was distilled under vacuum before use.  $\text{CDCl}_3$  was from Stohler Chemicals, U.S.A., containing 99% deuterated form. The NMR spectra were taken at 100 Mc/sec on the HA-100 NMR spectrometer of Varian, Inc., U.S.A. The samples were maintained at 31°. Tetramethylsilane (TMS) from Stohler Chemicals, U.S.A., was used as the reference compound except when pure DCA was used as the solvent; in the latter case, the lock was made on the CH-proton signal of DCA.

Figure 1 shows the NMR spectra of PSBC as a function of  $\text{CDCl}_3$ -DCA mixture composition. The bottom-most spectrum was obtained by weighing approximately 10 to 12 mg of the sample in the NMR sample tube, adding 0.01 ml of DCA to wet the sample and then 0.5 ml  $\text{CDCl}_3$  and mixing the contents. The other spectra were obtained by successively adding the required quantity of DCA to the solution and mixing. The top-most spectrum was obtained by dissolving the substance in pure DCA.

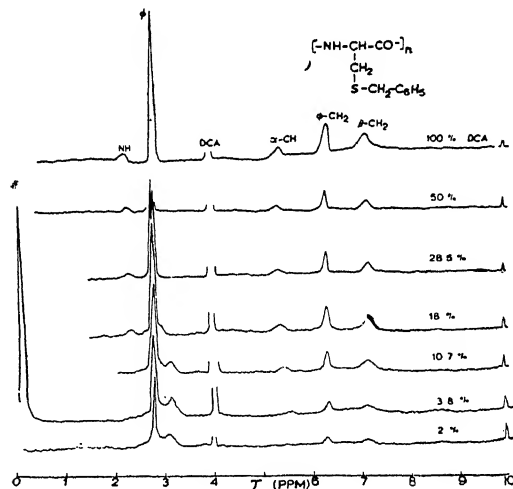


FIG. 1. Proton NMR spectra of PSBC at various  $\text{CDCl}_3$ -DCA mixtures. The internal reference is TMS.

The spectrum in 98% (v/v)  $\text{CDCl}_3$  solution is seen to consist of relatively broad peaks which can be assigned to the various protons as indicated in the figure. These assignments were made on the basis of available knowledge of NMR of polypeptides<sup>2</sup>. The peak due to the NH proton of the polypeptide backbone occurs at about 2.3 ppm and that of the  $\alpha$ -CH proton at about 5.6 ppm and are only barely visible at this composition of the solvent mixture. The peaks due to the  $\beta$ -CH<sub>2</sub> protons

at the sidechain is found to occur at 7.2 ppm while the benzylic protons appear at 6.3 ppm; the phenyl ring protons can be seen at about 3 ppm, close to the peak due to the proton impurity in  $\text{CDCl}_3$ .

As the DCA concentration in the mixture increases, all the peaks of PSBC are found to get sharper until about 25% DCA, when the spectrum is indistinguishable from that in pure DCA. Concurrent with the reduction in the linewidths of the peaks, one also observes a chemical shift of the peaks towards the downfield region (with respect to TMS).

The observed changes in the spectra with increasing DCA concentration can be interpreted as a conformational transition from a relatively highly ordered to a disordered state of the polypeptide on the basis of: (a) the relatively broad peaks in 98%  $\text{CDCl}_3$  as contrasted with the sharper ones in pure DCA indicating more mobility of the respective protons and (b) the downfield chemical shift of the peaks which is found to occur in the helix-coil transition in polypeptides<sup>3</sup>. That this transition could represent a  $\beta$ -structure  $\rightarrow$  coil transition is derived from the facts that (a) in compounds similar in structure to PSBC, such a transition in  $\text{CDCl}_3$ -DCA mixture has been reported<sup>2</sup> and (b) the ORD studies of Fraser *et al.*<sup>4</sup> on PSBC in ethylenedichloride-DCA mixture indicate the possibility of a  $\beta \rightarrow$  coil transition.

Further studies using ORD and UV spectral measurements to confirm our conjecture are being carried out and will be presented elsewhere<sup>5</sup>.

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## CRYSTAL DATA ON MONOBROMO-HYPOPHYLLANTHIN

As a part of a programme<sup>1,2</sup> of studying the structures of physiologically active compounds in this laboratory, the authors have taken up the structure of Monobromohypophyllanthin ( $\text{C}_{24}\text{H}_{29}\text{O}_7\text{Br}$ ). Figure 1 shows the structural formula of Monobromohypophyllanthin as given by L. R. Row and P. Satyanarayana. The composition<sup>3</sup> was determined by elemental chemical analysis (C, H, O, Br). This communication presents the crystal data of the substance.

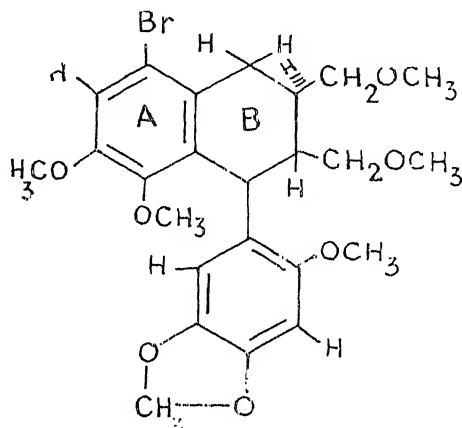


FIG. 1

The substance supplied by Prof. Ramachandra Row of the Andhra University, is crystallised by solution in methyl alcohol. The unit cell dimensions and space group of the crystal are determined from oscillation and Weissenberg photographs. The density of the crystal is determined by floatation in zinc chloride solution.

An examination of the Weissenberg photographs shows the following systematic absences only.  
 $h$  00,  $h$  odd; 0  $K$ O,  $K$  odd and 00 1, 1 odd.

This uniquely determines the space group of the crystal as  $P_{21212}$ . The crystal data are as follows:

Crystal data	
Chemical formula	$\text{C}_{24}\text{H}_{29}\text{O}_7\text{Br}$
Molecular weight	509.37
$a$	$= 28.80 \pm 0.02 \text{ \AA}$
$b$	$= 13.46 \pm 0.01 \text{ \AA}$
$c$	$= 6.06 \pm 0.01 \text{ \AA}$
Cell volume	$= 2349.15 \text{ \AA}^3$
Density calculated, $D_o$	$= 1.438 \text{ gm cm}^{-3}$
Density experimental, $D_x$	$= 1.430 \text{ gm cm}^{-3}$
Number of molecules in the unit cell	$= 4$
Crystal system	Orthorhombic
Space group	$P_{21212}$
$\mu$ for $\text{Cu K}\alpha$	$29.93 \text{ cm}^{-1}$

Further study on the crystal is in progress.

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#### PROTON-LIGAND STABILITY CONSTANTS OF SUBSTITUTED NAPHTHOLS IN 50% V/V ACETONE-WATER MIXTURE

THE presence of substituents at different positions in an organic molecule affects the pK value of its dissociable protons. The effects are generally additive and in most cases they are due to inductive, resonance and steric effects. A number of empirical relations<sup>1</sup> have been proposed in the past so that the prediction of an acid (or base) strength of an organic compound can be made with reasonable accuracy. Since pK values are required for the determination of metal ligand stability constants, we present in this paper the proton-ligand stability constants of 1-naphthol and some of the substituted naphthols. The proton-ligand stability constants were determined by potentiometric method using Bjerrum's method as modified by Chaberek and Martell<sup>2</sup>.

**Material and Methods.**—1-naphthol, 1-hydroxy-naphthalene 4-sulphonic acid (Na-Salt), 1-hydroxy 2-naphthoic acid and nitroso-R-Salt were obtained from Fluka Company. 2-nitroso 1-naphthol and 1-amino 2-hydroxynaphthalene 4-sulphonic acid were of B.D.H. quality. 1-Hydroxy 2-nitrososonaphthalene 4-sulphonic acid and 2-acetyl 1-naphthol were prepared by the reported literature methods<sup>3,4</sup>. Appropriate quantities of the reagents were dissolved in double distilled water and titrated potentiometrically. The acetone was of A.R. grade.

An ELICO pH Meter Model LI-10 with ELICO glass and Calomel electrodes capable of reading  $\pm 0.02$  was employed for the pH measurements. The pH meter was standardised with buffers of pH 4.00 and 9.00.

The pH titrations of the acids were conducted in a double wall jacketed glass cell through which water from a thermostat was circulated. The temperature within the cell containing dilute solution of the acid of known concentration (Ca.,  $2 \times 10^{-3}$  M) was maintained within  $\pm 0.1^\circ$  C. Once the solution attained the required temperature it was titrated by the addition of small amounts of standard alkali from a calibrated 5 ml micro-burette at a time and noting the pH. The ionic strength was kept constant by using a medium of 0.1 M  $\text{KNO}_3$ . Solutions of 1-hydroxy 2-naphthoic acid, 1-naphthol, 2-acetyl 1-naphthol, 1-hydroxy-naphthalene 4-sulphonic acid, 2-nitroso 1-naphthol, nitroso-R-Salt and 1-hydroxy 2-nitrososonaphthalene 4-sulphonic acid were prepared in 50% V/V acetone-water mixture using distilled water free from carbon dioxide. The pH values were corrected in all aquo-organic mixtures using the method of Van Uitert and Haas<sup>5</sup>.

**Results and Discussion.**—The proton-ligand stability constant of hydroxyl group of 1-naphthol and other substituted naphthols in 50% V/V acetone-water mixture at a constant ionic strength of 0.1 M  $\text{KNO}_3$  and  $35^\circ$  C are presented in Table I. The pK values of phenolic proton are in the following order.

1-Hydroxy 2-naphthoic acid > 1-naphthol > 2-acetyl 1-naphthol > 1-hydroxynaphthalene 4-sulphonic acid > 1-amino 2-hydroxy naphthalene 4-sulphonic acid > 2-nitroso 1-naphthol > nitroso-R-Salt > 1-hydroxy 2-nitroso naphthalene 4-sulphonic acid.

The introduction of electron withdrawing groups like nitroso, acetyl in ortho position and sulphonic in para position favours the easy liberation of phenolic proton in solution. As a result the pK value decreases in comparison with 1-naphthol. The higher value of 1-hydroxy 2-naphthoic acid is ascribed to intramolecular hydrogen bonding similar to salicylic acid<sup>11</sup>. The low pK value of 2-nitroso 1-naphthol in comparison with 1-hydroxy 4-sulphonic acid is due to the fall in inductive effect from ortho to para position.

In the case of 1-hydroxy 2-nitrososonaphthalene 4-sulphonic acid, the effects are additive due to the substitution in 2 and 4 positions. The predicted pK value is calculated as shown below:

decrease in pK for the substitution of nitroso group at 2-position =  $10.96 - 8.16 = 2.80$ .

decrease in pK for the substitution of sulphonic acid in 4-position =  $10.96 - 9.81 = 1.15$ .

expected decrease in pK for the substitution of nitroso at 2-position and sulphonic acid group at 4-position =  $2.80 + 1.15 = 3.95$ .

TABLE I

The proton-ligand stability constant of Hydroxyl group of 1-naphthol and other substituted naphthols at 35° C and an ionic strength  $\mu = 0.1 \text{ M KNO}_3$

Sl. No.	Name of the ligand	pK $\pm 0.02$ in 50 % V/V Acetone-water	Literature Value	Reference
1.	1-Hydroxy 2-naphthoic acid	.. 11.96	14.00 in 50 % dioxane water at 30° C	6
2.	1-Naphthol	.. 10.96	9.34 in aqueous medium at 25° C	7
3.	2-Acetyl 1-naphthol	.. 10.72	13.6 in 75 % dioxane-water mixture at 25° C	8
4.	1-Hydroxy naphthalene 4-sulphonic acid (Na-Salt)	.. 9.81	..	..
5.	1-Amino 2-hydroxy naphthalene 4-sulphonic acid	.. 8.46	..	..
6.	2-Nitroso 1-naphthol	.. 8.16	8.90 in 50 % dioxane water at 30° C	9
7.	Nitroso - R-Salt	.. 7.71	6.94 in aqueous medium	10
8.	1-Hydroxy 2-nitrosonephthalene 4-sulphonic acid	.. 7.16	..	..

Hence, the pK of 1-hydroxy 2-nitrosonephthalene 4-sulphonic acid will be  $(10.96 - 3.95 = 7.01)$ . The observed pK value of 7.16 for 1-hydroxy 2-nitrosonephthalene 4-sulphonic acid fairly agrees with the predicted one.

The low pK value in nitroso-R-Salt is due to the presence of two electron withdrawing groups in ortho position to hydroxyl group. The enhanced basic character in 1-amino 2-hydroxynaphthalene 4-sulphonic acid is due to the presence of electron repelling  $-\text{NH}_2$  group in ortho position and the electron withdrawing tendency of  $-\text{SO}_3\text{H}$  group. The electron withdrawing tendency of  $-\text{SO}_3\text{H}$  group will be much less in meta position when compared to para position.

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#### RESACETOPHENONE OXIME AS AN INDICATOR FOR THE TITRIMETRIC DETERMINATION OF FLUORIDE

ANALYTICAL applications of resacetophenone oxime have been reported earlier from these laboratories<sup>1-4</sup>. In the present communication the application of this compound in the titrimetric determination of fluoride is described.

Resacetophenone oxime reacts with iron (III) in the pH range 4.5-7.0 to form a violet colored (1:1) complex<sup>3</sup>. Ferric fluoride complex is stronger than ferric resacetophenone oxime complex and this forms the basis for the procedure described below.

**Procedure.**—A known volume of sodium fluoride was taken in a 100 ml pyrex conical flask, about 2.0 gm of sodium chloride was next added. The

contents were shaken well and treated with an equal volume of 95% ethanol, followed by 1 ml of the indicator solution (2% solution in 95% ethanol). It was then titrated with 0.05 N ferric chloride solution in 0.01 hydrochloric acid; the appearance of a permanent light violet color indicated the end point. The typical results are recorded in Table I.

TABLE I

Determination of fluoride with resacetophenone oxime as indicator

S.No.	Fluoride, mg		error %	
	taken	found		
1	..	5.29	5.26	-0.56
2	..	7.40	7.42	+0.35
3	..	10.58	10.52	-0.56
4	..	11.63	11.61	-0.13
5	..	12.69	12.70	+0.04
6	..	13.75	13.64	-0.80
7	..	14.81	14.71	-0.67
8	..	15.87	15.95	+0.50

**Interference.**—The elements usually associated with fluoride, viz., chloride, iodide, bromide, borate, phosphate, perchlorate, silicate, nitrate, sulphate, nitrite, carbonate, sulphite, tartrate, oxalate, citrate, and acetate were added in 10 fold excess in each case and the titration was carried out using the same procedure. Amongst these ions borate, phosphate, silicate, tartrate, acetate, citrate, oxalate and sulphite caused interference by giving rise to premature end point. Nitrite interfered only when it was present in more than 2 fold excess. Carbonate altered the pH and thus interfered; elimination of carbonate with dilute hydrochloric acid is necessary prior to the commencement of the titration.

**Discussion.**—Tables I and II clearly show that fluoride could be satisfactorily determined by this method when present alone and also in the presence of 10 fold excess of certain ions which are usually associated with fluorine in waters and ores.

Resacetophenone oxime can be easily prepared and recrystallised from aqueous alcohol using animal charcoal. The alcoholic solutions are fairly stable for long periods. The authors therefore consider this as a good indicator for the determination of fluoride with iron (III). Thiocyanate<sup>5</sup> and sodium salicylate<sup>6</sup> were used as indicators in the determi-

TABLE II  
Effect of foreign ions in the determination of fluoride

Fluoride taken, mg	Foreign ions added (10-100 mg)	Fluoride found mg	Error %
10.35	nitrate (NaNO <sub>3</sub> )	10.37	+0.19
10.35	sulphate (Na <sub>2</sub> SO <sub>4</sub> )	10.37	+0.19
10.35	chloride (NaCl)	10.37	+0.19
10.35	Iodide (KI)	10.37	+0.19
10.35	bromide (KBr)	10.37	+0.19
10.35	carbonate (Na <sub>2</sub> CO <sub>3</sub> )	10.37	+0.19
10.35	perchlorate (KClO <sub>4</sub> )	10.37	+0.19

nation of fluoride with iron (III). Color change in the case of thiocyanate is not facile. sodium salicylate is a sensitive indicator, but it is necessary to maintain carefully controlled conditions for this titration. In the case of resacetophenone oxime the little acid that is usually employed in the preparation of ferric chloride (0.01 N HCl) will form the necessary pH conditions for the titration. Hence it is a simple and rapid method.

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#### SYNTHESIS OF THE N-TERMINAL TETRAPEPTIDE SEQUENCE OF HUMAN FIBRINOPEPTIDE-A

FIBRINOPEPTIDE-A is released from fibrinogen during the process of blood coagulation<sup>1</sup>. Human fibrinopeptide-A is a hexadecapeptide with the amino acid sequence<sup>2</sup>, Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-  
1 2 3 4 5 6 7 8 9  
Ala-Glu-Gly-Gly-Gly-Val-Arg. With a view to synthesise this hexadecapeptide by the fragment condensation, the two protected hexapeptides Boc-Glu (OBzl)-Gly-Gly-Gly-Val-Arg (NO<sub>2</sub>)-OMe\*



corresponding to positions 11–16 and Boc-Glu (OBzl)-Gly-Asp (OBzl)-Phe-Leu-Ala-OMe corresponding to positions 5–10 have been synthesised in our laboratory<sup>3,4</sup>. We now wish to report the synthesis of the third fragment, the N-terminal tetrapeptide sequence, Ala-Asp-Ser-Gly.

The reaction of Z-Ala-OPCP<sup>5</sup> with Asp (OBzl)<sup>6</sup> yielded Z-Ala-Asp (OBzl) in 81.8% yield, m.p. 140–142°,  $[\alpha]_D^{23} + 17.38^\circ$  (DMF; c. 1.5) (Found: C, 61.67; H, 5.63; N, 6.52%.  $C_{22}H_{24}N_2O_7$  requires C, 61.67; H, 5.61; N, 6.54%). This was converted to the pentachlorophenyl ester, Z-Ala-Asp(OBzl)-OPCP, m.p. 175–178°, in 75% yield using the DCCI procedure. This active ester can also be obtained in 48% yield by the condensation of Z-Ala<sup>7</sup> with Asp (OBzl)-OPCP using the mixed anhydride method. Reaction of Z-Ala-Asp (OBzl)-OPCP with Ser-Gly (obtained by the catalytic hydrogenation of Z-Ser-Gly-OBzl<sup>8</sup>) led to the tetrapeptide Z-Ala-Asp (OBzl)-Ser-Gly, yield, 51%, m.p. 125–128°,  $[\alpha]_D^{23} + 10.5^\circ$  (DMF; c. 0.95) (Found: C, 52.2; H, 5.7; N, 8.97%.  $C_{27}H_{32}N_4O_{10} \cdot 3H_2O$  requires C, 51.90; H, 5.80; N, 8.94%). This protected tetrapeptide can also be obtained by an alternate method in which Boc-Asp (OBzl)-OPCP was reacted with Ser-Gly to furnish Boc-Asp(OBzl)-Ser-Gly, yield, 37%, m.p. 112–113°,  $[\alpha]_D^{23} - 22.3^\circ$  (DMF; c. 2.0) (Found: C, 53.98; H, 6.25; N, 8.99%.  $C_{21}H_{29}N_3O_9$  requires C, 53.94; H, 6.25; N, 8.98%). After deprotection of this tripeptide with HCl in ethyl acetate the product was made to react with Z-Ala-OPCP to yield Z-Ala-Asp (OBzl)-Ser-Gly in 51.5% yield.

A superior method of obtaining this tetrapeptide involved the conversion of Z-Ala-Asp (OBzl) to the active ester, Z-Ala-Asp (OBzl)-OSu using the DCCI procedure, yield, 85%, m.p. 105–108°,  $[\alpha]_D^{23} - 19.61^\circ$  (DMF; c. 2.0) (Found: C, 60.37; H, 5.11; N, 8.10%.  $C_{26}H_{27}N_3O_9$  requires C, 59.99; H, 5.18; N, 7.99%), followed by its condensation with Ser-Gly. The Product, Z-Ala-Asp (OBzl)-Ser-Gly, obtained in 71% yield, on catalytic reduction over 10% Pd-C in acetic acid, gave Ala-Asp-Ser-Gly AcOH, yield, 63.5%, m.p. 190–194°,  $[\alpha]_D^{23} + 31.7^\circ$  (1N HCl; c. 0.63) (Found: C, 41.09; H, 6.44; N, 13.04%.  $C_{14}H_{24}N_4O_{10}$  requires C, 41.16; H, 5.92; N, 13.72%).

The tetrapeptide sequence Ala-Asp-Ser-Gly is also present in the enzyme staphylococcal nuclease<sup>9</sup> and the dipeptide sequence -Asp-Ser- is present at the catalytic site of several proteolytic and hydrolytic enzymes like trypsin, chymotrypsin and cholinesterase<sup>10</sup>.

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\* The amino acids used, with the exception of glycine, have the L-configuration. Abbreviations: Z, benzyloxycarbonyl; Boc, *tert*-butoxycarbonyl; OBzl, benzyl ester; OPCP, pentachlorophenyl ester; OSu, N-hydroxysuccinimide ester; DCCI, dicyclohexyl carbodiimide.

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### STABILITY OF AQUEOUS CHLORAMINE-T SOLUTIONS TO HEAT AND ULTRA-VIOLET RADIATION

CHLORAMINE-T ( $p\text{-CH}_3\text{-C}_6\text{H}_4\text{SO}_2\text{NClNa} \cdot 3\text{H}_2\text{O}$ ), the sodium salt of *p*-toluene sulfochloramide has been used as a mild oxidant in volumetric analysis and as a chlorinating agent. Although the stability of aqueous solutions of chloramine-T (CAT) has been critically examined by several workers, there are controversial reports and these are summarized by Bishop and Jennings<sup>1</sup>. Photochemical decomposition of CAT solutions with polychromatic radiations has been reported by Eisenschimmel<sup>2</sup> and Carlsen<sup>3</sup>, but no conclusive data on the mechanism are available. In the present work, some preliminary studies have been made on aqueous CAT solutions, (i) about their thermal stability over the temperature range 50–95° and (ii) on their photochemical stability at 3660 Å.

*Experimental.*—Chloramine-T (Rhodia) was purified by the method of Morris *et al.*<sup>4</sup>. An approximately decimolar stock solution was prepared and was standardized by the iodometric method. Reagent grade materials were used in preparing solutions of other compounds. All solutions were prepared in triply distilled water.

Thermal decomposition studies were carried out with aliquots of 0.053 M CAT solutions (25 or

50 ml) in iodine flasks, in a thermostatically controlled water-bath, over a period of 1-4 hours.

UV irradiations were carried out with (i) a stabilized Hanovia (Fluorescence lamp model II) U-shaped, 125 Watts quartz mercury arc lamp and (ii) Philips medium pressure mercury vapor lamps (80 and 250 W). A Woods filter was used to obtain monochromatic radiation at 3660 Å. Exactly 10 ml of CAT solution were taken in Pyrex cells of path length 20 mm, fitted with glass stoppers. Irradiations were carried out in a typical photochemical reaction cell assembly whose temperature was maintained at 30° with the help of an ultra thermostat. Intensity measurements were made using uranyl oxalate actinometer. The intensity of absorbed light  $I_a$ , was determined using the relation  $I_a = I_0 F$ , where  $I_0$  is the incident intensity and  $F$  is the fraction of light absorbed<sup>5</sup>. A Beckman DB spectrophotometer was used for recording the UV spectra of CAT solutions. pH measurements were made on a Elico model Li-10 pH meter.

The extent of thermal and photochemical decomposition of CAT solutions was determined by iodometric titration of the experimental solution and comparison with a blank solution of the compound.

**Results and Discussion.**—It was observed that aqueous solutions of CAT (0.053 M) are stable at 50° up to a heating period of 3 hours. At the end of 4 hours, a slight decomposition of 0.255% was noted. At 60°, the solution was stable for one hour, but at the end of two hours, decomposition was 0.255% which then became constant. At 70°, the solution showed a decomposition of 0.255% even at the end of one hour which became constant. At 80°, the decomposition of 0.255% observed after one hour increased to 1.104 at the end of four hours, while at 95° it increased from 1.274 to 5.772. No free chlorine was evolved from the solutions and the pH dropped from 8.35 to 7.9 on boiling the solution for about 20 minutes.

Photochemical decomposition of CAT solutions (0.0919-0.00284 N) was found to obey a first order rate law. The rate constant  $k$  increases with dilution from  $1.99 \times 10^{-3}$  to  $15.28 \times 10^{-3} \text{ min}^{-1}$  at  $I_0 = 8.56 \times 10^{18} \text{ quanta min}^{-1}$ . The quantum efficiency  $\gamma_{\text{net}}$  for the disappearance of CAT is around 0.20 and experiments at 40° showed that the temperature coefficient is 1.1. Further, a plot of  $\log R = kc$  where  $c$  is the concentration of CAT (moles l<sup>-1</sup>) Vs  $\log I_a$  gave a straight line with slope around unity. A slight decrease in  $k$  was noticed by the addition of NaCl to the solution. pH measurements showed that solutions of CAT become more acidic upon irradiation and a slightly yellow coloration was noticed. However, no free

chlorine could be detected. UV spectrum of CAT consists of a broad absorption band with a maximum around 300 nm ( $\epsilon_{\text{max}} = 44.0$ ) and extending into the vacuum UV region. No apparent change was noticed in the spectrum upon irradiation.

The thermal instability of CAT solutions at elevated temperatures could be ascribed to the loss of positive chlorine thereby decreasing the iodometric titer. Rao *et al.*<sup>6</sup> have noticed significant decrease in iodometric titer of CAT solutions at pH 2.65-5.65 when acidified with mineral acids. This has been attributed to the minor side reactions leading to the formation of  $\text{Cl}_2$  and the dimer  $\text{R}_2\text{N}_2\text{Cl}_2$ , the former escaping from the solution. (R stands for  $\text{CH}_3-\text{C}_6\text{H}_4\text{SO}_2$ ), during the disproportionation of monochloramine-T ( $\text{RNHCl}$ ), into  $\text{RNH}_2$  and  $\text{RNCl}_2$ . The dimer  $\text{R}_2\text{N}_2\text{Cl}_2$  does not liberate iodine from KI solutions. In the present investigations no free chlorine was detected and formation of  $\text{R}_2\text{N}_2\text{Cl}_2$  requires the formation of the precursor  $\text{RNCl}^\circ$  radical from an electron elimination reaction. Bishop and Jennings<sup>1</sup> have pointed out that an aqueous solution of CAT contains several species such as  $\text{RNHCl}$ ,  $\text{RNCl}_2$ ,  $\text{RNH}_2$ ,  $\text{HOCl}$  and  $\text{OCl}^-$  ion, out of which all the species except  $\text{RNH}_2$  oxidize KI solution. While  $\text{RNHCl}$  is not isolated due to its rapid disproportionation into  $\text{RNCl}_2$  and  $\text{RNH}_2$ , the most likely unstable species in a solution of CAT are  $\text{HOCl}$  and  $\text{OCl}^-$  ion. Bishop and Jennings<sup>1</sup> have shown that concentration of  $\text{OCl}^-$  ion increases with increase in pH while that of  $\text{HOCl}$  is constant. It has been shown that  $\text{HOCl}$  and  $\text{OCl}^-$  ion would be the reacting species in some of the oxidation reactions<sup>7-9</sup> brought about by CAT. We propose that the reduction in iodometric titer of CAT solutions on heating could be due to the decomposition of these unstable species, with loss of oxygen. We conclude that oxidation reactions with CAT solutions should not be carried out at elevated temperatures.

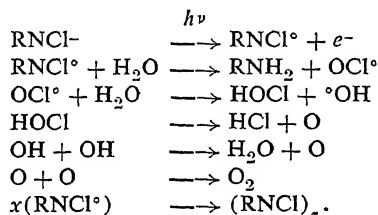
Photochemical decomposition of CAT solution vaguely resembles the photolysis of  $\text{OCl}^-$  ion. The latter has been studied by a number of workers<sup>10,11</sup>. The decomposition of  $\text{OCl}^-$  ion follows a monomolecular course, with  $\text{O}_2$ ,  $\text{Cl}^-$ ,  $\text{ClO}_2^-$  and  $\text{ClO}_3^-$  ions as the photolytic products<sup>11</sup>. In the present work, photolysis of CAT solutions can be represented by the equation :

$$-\frac{d(\text{CAT})}{dt} = k I_a (\text{CAT})$$

where (CAT) indicates the concentration of CAT solution. It is likely that  $\text{OCl}^-$  ion gets photolysed in a solution of CAT.

An attempt was made to identify the photolytic products. Irradiations for 30-60 minutes produced

only an yellow coloration but when the solution was irradiated for longer periods (4-6 hours) a pale yellow precipitate was obtained. This was filtered, washed with water. When the product was extracted with alcohol or benzene an insoluble residue was left behind, but the product was completely soluble in pyridine and tetrahydrofuran. Attempts to check the homogeneity of the precipitate by TLC and paper chromatographic techniques with benzyl alcohol saturated with water as the solvent showed that the product consisted of at least two fractions. The pyridine solution was found to be unstable giving 4-5 fractions. These results agree with those of Dietzel and Taufel<sup>12</sup> who photolysed aqueous CAT solutions with polychromatic radiation and obtained a complex mixture containing acidic and basic products. In the present work, it is likely that the photolysis of CAT could lead to the decomposition of HOCl and formation of resinous products (RNCl)<sub>x</sub> as follows:



However, a complete analysis of the photolytic product (or products) would become necessary for proposing a detailed mechanism for the photochemical decomposition of aqueous CAT solutions.

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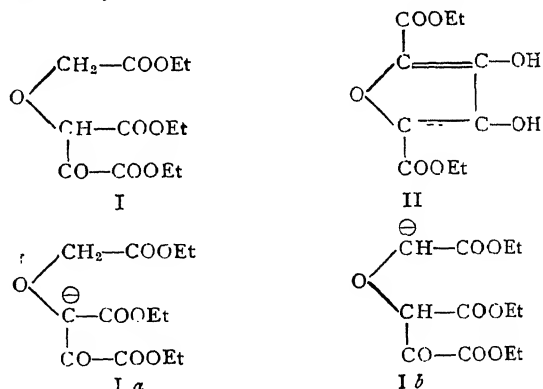
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### MECHANISM OF THE DIECKMANN-KOMPPA REACTION OF DIETHYL OXALATE WITH DIETHYL OXYDIACETATE

THE Dieckmann-Komppa reaction of diethyl oxalate and diethyl oxydiacetate to yield 2,5-dicarbethoxy-3,4-dihydroxyfuran (II) has been effected earlier<sup>1-3</sup>; but a plausible intermediate, formed by the initial Claisen condensation of the esters, had not been isolated.

This intermediate, triethyl  $\alpha$ -oxalyl oxydiacetate (I), is formed in about 60% yield when diethyl oxalate and diethyl oxydiacetate are condensed in molar quantity of ethanol-free sodium ethoxide in ether at 0°; a 15% yield of the furan derivative (II) is also obtained.

It was observed that the oxalyl intermediate (I) gave extremely low yields of the furan (II) when its cyclisation was tried with sodium in refluxing benzene, or ethanol-free sodium ethoxide in ether. However, when the experimental conditions were altered to refluxing ethanolic sodium ethoxide, excellent yields of the furan (II) were obtained.



Similarly, in the Dieckmann-Komppa reaction of diethyl oxalate and diethyl oxydiacetate, it was observed that the best yields were obtained when the reaction was conducted in the presence of molar quantity of ethanolic sodium ethoxide. Even with excess of refluxing ethanolic sodium ethoxide, the furan (II) was recovered quantitatively, indicating no reversal of the reaction.

It is suggested<sup>4</sup> that in the presence of a low concentration of ethoxide ions as with ethanol-free sodium ethoxide or sodium dust, the carbanion Ia is formed by abstraction of the most acidic hydrogen atom and the reaction does not proceed further as the insoluble sodium salt separates. Whereas, with ethanolic sodium ethoxide, in the presence of large concentration of ethoxide ions, the carbanion Ib is also formed which under equilibrating reaction conditions leads to the furan derivative (II).

#### Experimental

*Triethyl  $\alpha$ -oxaloxycydicetate (I).*—To ethanol-free sodium ethoxide, from 1.2 g sodium, in ether (50 ml), diethyl oxalate (7.3 g) was added and the resultant yellow solution cooled in ice. A solution of diethyl oxycydicetate (9.5 g) in ether (25 ml) was carefully added to the above solution and the reaction mixture kept at 0° for 72 hr. On working up<sup>4</sup> (the oxalyl derivative was soluble in water and hence the aqueous phases had to be saturated with sodium chloride and repeatedly extracted with ether), triethyl  $\alpha$ -oxaloxycydicetate (I) (8.2 g, 57%) and 2, 5-dicarbethoxy 3, 4-dihydroxyfuran (II) (1.8 g, 15%), m.p. 188°, were obtained. The oily oxalyl derivative (I) gave intense violet colour with ferric chloride solution and on pyrolysis, it evolved carbon monoxide. It was used for cyclisation without further purification as it decomposed on vacuum distillation.

*Cyclisation of Triethyl  $\alpha$ -oxaloxycydicetate (I).*—To refluxing sodium ethoxide, from 0.92 g sodium in ethanol (50 ml), was added dropwise a solution of triethyl  $\alpha$ -oxaloxycydicetate (I) (5.8 g) in ethanol (25 ml) over 5 min. and the reaction mixture refluxed for 5 hr. Excess of ethanol was removed by distillation under vacuum and the reaction mixture, on working up, yielded 2, 5-dicarbethoxy-3, 4-dihydroxyfuran (II) (3.3 g, 68%), m.p. 181–4°. On recrystallisation from ethanol, it melted at 188°; lit.<sup>2</sup>, m.p. 189°.

With sodium dust in refluxing benzene, under identical conditions, the yield of furan (II) was only 10%.

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#### VARIETAL DIFFERENCE ON THE NUTRIENT COMPOSITION OF BANANA POWDERS

THE edible portion of banana fruit includes water, carbohydrates, fats, proteins, organic acids, mineral matters and other volatile constituents. The proportion in which these constituents occur vary greatly in different varieties of bananas. The present study reports the nutrient composition of Basrai, Harichal, Lalkel (variety of *Musa cavendishii*), Rajeli, Safed Velchi (variety of *Musa paradisiaca*) banana powders.

Basrai banana was obtained from Jalgaon District, Maharashtra State, while other varieties of bananas were obtained from Bassein Road, Bombay. In order to get banana bunches of uniform maturity, nearly 100 banana plants were tagged at the time of inflorescence emergence in a nearby banana plantation where uniform cultural practices were maintained throughout the growing season. From the above lots two bunches each of uniform development were harvested at 100 days of growth (full round shape) after the inflorescence emergence. These bunches were immediately brought to the laboratory, separated into hands and stored in an incubator at a temperature of 18–20° C.

Banana powder was prepared in the following way:

Raw bananas of the above five varieties were allowed to ripen in the laboratory, in an incubator at a temperature of 18–20° C and a relative humidity of 68–75%. The ripening was allowed to proceed to a stage when the fruit became very soft. The fruits were peeled and pulp was then cut into small pieces with the help of stainless steel knife. The macerated pulp was then dried at 60° C under 58 cm of vacuum. After nine hours, the dried product was pulverised and passed through 50 mesh sieve and the powder was stored in air tight polyethylene bags in air tight container. These banana powders were analysed for proximate principles, minerals and vitamins.

Proximate principles and calcium were estimated by A.O.A.C., method<sup>1</sup>. Total titratable acidity was determined according to the method suggested by Miller<sup>2</sup>. Pectin as calcium-pectate was determined by the method of Carre-Haynes<sup>3</sup> as modified by Joslyn<sup>4</sup>. Total sugar percentage was calcu-

TABLE I  
 "Proximate composition of banana powders"  
 (All values expressed as gm per 100 gm banana powder)

Variety	Moisture	Ash	Fat	Protein N × 6.25	Total sugars	Crude fibers	Starch	Pectin as pectate	Total acidity as citric acid
Basrai	..	1.39	2.55	0.62	2.65	80.58	2.22	3.17	3.54
Harichal	..	2.57	2.25	0.44	2.65	77.74	1.99	4.89	3.05
Lalkel	..	1.38	2.48	0.41	2.18	79.71	2.33	3.05	3.76
Rajeli	..	1.95	2.30	0.49	2.53	82.43	1.79	2.63	2.56
Safed Velchi	..	1.05	2.62	0.60	2.38	82.64	2.20	1.85	2.87

TABLE II  
 "Mineral composition of banana powders"  
 (All values expressed as mg per 100 gm)

Variety	Calcium	Iron	Phosphorus	Magnesium	Sodium	Potassium	Silica
Basrai	..	26.81	19.0	194.4	46.53	208.00	68.6
Harichal	..	18.78	7.0	120.4	47.91	270.21	59.8
Lalkel	..	27.21	9.5	134.0	58.53	210.32	83.7
Rajeli	..	23.21	7.2	138.6	48.02	294.32	76.3
Safed Velchi	..	20.43	16.0	181.6	55.79	334.70	74.3

lated by the difference (100 sum of the percentage of all the other components). Phosphorus was estimated by the hydroquinone reduction method of Bell and Doisy<sup>5</sup> as modified by Sterges *et al.*<sup>6</sup>. Iron was estimated from the ash solution of banana powder by the Colorimetric method of Elvehjem<sup>7</sup>. Magnesium was estimated by Dickinson's method<sup>8</sup>. Sodium and Potassium were estimated by flame photometer. Silica and Sand (insoluble residue in HCl) was determined according to A.O.A.C. method<sup>1</sup>. Thiamine was estimated by Jansen's Thiochrome method<sup>9</sup> as suggested by Harris and Wang<sup>10</sup>. Riboflavin was estimated by Scott *et al.* method<sup>11</sup>, niacin by the method of Swaminathan<sup>12</sup> and total apparent ascorbic acid by Bessey and King method<sup>13</sup>.

It can be seen from Table I that total sugar content was relatively high in these samples. Protein content of these varieties was low than the protein content of other varieties reported by different workers<sup>14,15</sup>. Total ash content was remarkably higher than that of other varieties reported by

Simmonds<sup>16</sup> and Adriaens<sup>17</sup> and comparable to that reported by Spoon<sup>18</sup>. Crude fiber ranged from 1.79–2.22 g. per cent in banana powders. The starch content of these varieties was significantly lower than starch content of Poovan variety reported by Subrahmanyam<sup>15</sup>. The changes in pectin content as calcium pectate was not appreciable in samples studied and varied from 3.28–4.50 g per cent. Total acidity content of all samples was found to be higher than the total acidity in different species of banana powder<sup>15</sup>.

Table II gives the mineral content of banana powders. The calcium content of these varieties ranged from 18.78–27.21 mg per cent, and iron content 7.0–19.0 mg per cent respectively. With respect to calcium and iron contents, these varieties were almost comparable to Poovan variety banana powder<sup>15</sup>. Basrai banana powder had highest amount of calcium and iron among the samples studied. Phosphorus and magnesium were present in appreciable amounts. It would be however interesting to elucidate the mineral contents that

more than 50% of ash was potassium. The high amount of potassium suggest the use of banana powder in condition of hypertension and liver disease<sup>10</sup>. Sodium content varied from 208–334.70 mg per cent in the samples.

The vitamin contents of banana powders are reported in Table III. Different varieties of banana powders differed in their contents of B-group vitamins and ascorbic acid. Thiamine content of Safed Velchi was found to be highest (94 µg %). Riboflavin content in the samples varied from 29–79 µg per cent. Niacin was found within a range of 610–920 µg per cent. Ascorbic acid ranged in amounts from 15.12–21.40 mg per cent. Safed Velchi and Lalkel banana powders were rich in ascorbic acid.

TABLE III

*Thiamine, riboflavin, niacin and ascorbic acid content of banana powders*

Variety	Thia- mine B <sup>1</sup> µg %	Ribo- flavin µg %	Niacin µg %	Total apparent Ascorbic acid mg%
Basrai	83	34	730	16.94
Harichal	80	29	610	15.12
Lalkel	88	52	860	19.73
Rajeli	72	36	790	18.46
Safed Velchi	94	79	920	21.40

On the whole, the banana powders were found to contain many of the nutrients that were normally required for the general well being of the body. Of the five varieties examined, Safed Velchi, Basrai and Lalkel banana powders showed fairly good percentage of minerals and vitamins.

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## MINERAL NUTRIENT VALUE OF CITRUS FRUITS

FRUITS are one of the main sources of vitamins and minerals<sup>1-3</sup>. These two nutrients, in minute quantities, are essential for growth and disease resistance. Many suffer from deficiencies of vitamins and minerals, and liberal inclusion of fruits will be helpful. Even cheap fruits like the Citrus fruits are in fact more 'nutritious than' the expensive apple. Hence it will be interesting to study the mineral contents of various Citrus fruits and compare them so that it will be easier for an individual to understand the nutritive merit of the different Citrus fruits.

Five varieties of Citrus fruits from Kollimalai region of Trichy District are chosen for this investigation. They are analysed quantitatively for their moisture content, ash content, and mineral content<sup>4</sup>.

*Procedure.*—A sample of edible portion (juice sac with juice), of Citrus fruits for each variety is obtained and its moisture content is determined by oven drying to constant weight at 70° C. The dried sample is ground and ignited at low red heat to produce the ash quantitatively. About 0.4 gram of the ash is digested with 10 ml of 1:1 HCl to a paste twice and then extracted with water containing a little of 1:1 HCl. The solution is then made up to 250 ml with distilled water. This solu-

TABLE I

Name of the Citrus Fruit	Botanical Name	Moisture content	Ash content	Minerals Present
1. Lime	<i>Citrus aurantifolia</i>	84.23	0.6404	Na, K, Mg, Ca, P, Zn, Fe, Al, S, Cl
2. Kamala Orange	<i>Citrus reticulata</i>	85.92	0.4139	„ „
3. Orange	<i>Citrus sinensis</i>	89.2	0.4860	„ „
4. Kolunji	<i>Citrus limettoides</i>	85.39	0.5919	„ „
5. Kadarangai	<i>Citrus medica</i>	85.43	0.6193	„ „

TABLE II

Name of the fruits	Weights in mgms per 100 grams of the edible portion of the fruits					
	Ca	Fe	P	Na	K	Mg
1. Lime	95.54	0.4812	19.86	1.88	58.35	14.92
2. Kamala Orange	55.33	0.1937	18.41	1.614	58.76	10.15
3. Orange	46.70	0.2390	17.62	1.866	56.14	13.48
4. Kolunji	64.91	0.3398	26.34	1.912	78.16	16.00
5. Kadarangai	86.08	0.3438	26.40	1.719	77.34	20.06

tion is used for qualitative analysis and the minerals present in each variety are noted (see Table I).

All the elements present, except Al, S, Cl and Zn are then estimated quantitatively. The weight of Ca present in the ash is determined volumetrically by precipitating calcium as oxalate and titrating it with standard solution of potassium permanganate in presence of 1:1 sulphuric acid. Iron is estimated colorimetrically as  $[\text{Fe}(\text{CNS})_6]^{3-}$ , using Systronics Double cell colorimeter<sup>5</sup>. With the same instrument, the weight of phosphorus is determined by the "Molybdenum blue method".

The elements sodium and potassium are estimated flame photometrically with Bruno-Lange (filter type) Flame photometer. For these estimations, petrol gas is used as the fuel with a pressure of 0.65 kg per  $\text{cm}^2$ . Magnesium is estimated by titrating it with 0.01 mole EDTA at pH 10 using Erio-T indicator. The interference of other elements is suppressed by adding a little of potassium cyanide and hydroxyl amine hydrochloride. From the total volume of EDTA consumed the volume equivalent of calcium present is subtracted and the resultant volume of EDTA gives the weight of magnesium present.

The weights of minerals thus calculated are then expressed in milligrams per 100 grams of the edible portion of the fruits (see Table II).

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AMINO ACIDS IN ROOT EXUDATES OF  
HEALTHY AND *HELMINTHOSPORIUM*  
*TURCICUM* INFECTED SORGHUM PLANTS

It is well known that micro-organisms in the rhizosphere receive nutrition from the 'root exudates' which are mainly low molecular substances<sup>1</sup>. These substances readily available to micro-organisms are of great importance for the population of plant root surfaces and are, from all aspects, the determining factors of rhizosphere effect<sup>2</sup>.

Studies on changes in rhizosphere microflora due to pathogenic condition in plants is receiving increased attention. This is understandable because any diseased condition in plants results in changed physiology and such changes cause alterations in the chemical composition of root exudates, resulting in altered rhizosphere effect<sup>3</sup>. In the present investigation attempt was made to study the influence of pathogenicity on the quantitative-qualitative occurrence of amino acids in root exudates of sorghum.

Seedlings of CO. 18 sorghum strain (*Sorghum subglabrescens*) were raised in a root exudation apparatus adopting the method and apparatus developed by Balasubramanian and Rangaswami<sup>4</sup>. Plants were inoculated with spore suspension (10,000 spores/ml) of *Helminthosporium turcicum* Pass, causing leaf blight disease of sorghum, on the 15th day and after a fortnight the root exudates from the inoculated plants were pooled and analysed for amino acids. Exudates from healthy plants were also analysed simultaneously for comparison. The exudates so collected after desalting were analysed for amino acids by passing through ion-exchange resin columns (Dowex-1 and Dowex-50 resins) following a modified procedure of Husain and McKen<sup>5</sup>. Pyridine (0.1 M) was used to elute amino acids from Dowex-50, *n*-butanol acetic acid-water (4:1:1 v/v) and phenol-water (3:1 v/v) solvent systems were employed in two-dimensional ascending chromatographic method for separation and identification of amino acids. Ninhydrin positive spots were quantitatively estimated by the method of Demetriades<sup>6</sup>.

It is evident from Table I that while the concentration of asparagine, aspartic acid, cysteine/cystine, glutamic acid, leucine(s), methionine, proline, serine and threonine reduced, there was increase in the concentrations of alanine, histidine and phenylalanine in the root exudates of diseased plants as compared to that in the healthy plants. In addition, unlike in healthy plants, the root exudates of infected plants did not contain glycine, lysine and valine. Thus the present results conclusively prove that disease incidence which results in

TABLE I

A comparison of the amino acids present in the root exudates of healthy and diseased sorghum plants (Concentration expressed in  $\mu\text{g/plant}$ )

Amino acids	*A	B
Asparagine	.. 270	120
Aspartic acid	.. 320	210
Alanine	.. 110	130
Cysteine/Cystine	.. 120	80
Glutamic acid	.. 480	320
Glycine	.. 70	..
Histidine	.. 80	120
Leucine(s)	.. 100	60
Lysine	.. 90	..
Methionine	.. 120	70
Phenylalanine	.. 140	170
Proline	.. 230	130
Serine	.. 260	190
Threonine	.. 280	150
Tryptophan	.. 170	200
Tyrosine	.. T	60
Valine	.. T	..
Unidentified	.. 1	2
Total concentration	.. 2,840	2,010

\* A — Amino acids in the root exudates of healthy plants. B — Amino acids in the root exudates of diseased plants.

— Not detectable.

T Present in trace.

pronounced changes in physiology of host, could bring both quantitative and qualitative changes in the root exudation pattern. The significance of decreased concentration of certain amino acids like asparagine, glutamic acid, glycine and leucine(s) and other sulphur containing amino acids in root exudates of diseased plants deserves some attention as this would greatly affect the rhizosphere microflora.

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#### AFFINITY OF SHEEP POX VIRUS (SPV, STRAIN) FOR HETEROLOGOUS SYSTEMS

ON reviewing the literature on sheep pox it has been observed that workers have mostly attempted to cultivate sheep pox virus in homologous system in the hope to produce vaccine virus for large scale immunization of sheep. Evidently, heterologous system need to be investigated in greater detail to that virulence is lowered; therefore, this study was undertaken to passage sheep pox virus in different heterologous systems.

Sheep pox virus (SPV) was received as infected skin of lambs suspended in buffered glycerine saline from the department of bacteriology of U.P., Vety. College, Mathura. The virus was purified by Arcton according to the method of Epstein (1958).

##### Tissue Culture

Mouse embryo fibroblast monolayers were prepared, according to the method of Evans and Salaman (1965) except we used inactivated lamb serum.

Chick embryo fibroblast were prepared from 10-day-old white leghorn embryonated eggs. Cell preparation was same as in mouse embryo except tissue was trypsinised for 40 min. and number of cells was adjusted to 300,000/ml.

##### Inoculation in Unirradiated Animals

The SPV (10,000 SID 50) was inoculated intracranially in 4-day-old mice and rats and intratesticularly in 20, 40, 60-day-old male mice and rats at the rate of 0.03 ml/animal. After 6 days of observation the animals were sacrificed and such 6 and 4 blind passages were given respectively.

Rabbits and guinea pigs (6 months old) were given, in each testis, 0.5 ml of SPV (10,000 SID 50). The testis and animals were observed for 6 days. Such four successive passages were given. The sheep pox virus (10,000 and 100 SID 50) was inoculated in 12-day-old embryonated eggs by chorio-allantoic (CAM) route at the rate of 0.2 ml/egg. After 96 hours at 37°C virus was harvested and such 4 blind passages were given each time the CAM was examined visually.

##### In Irradiated Mice

X-Ray irradiation. A200 KV-X-Ray apparatus (Siemens) with an irradiation rate of 200 r/

3 mins. 10 sec., of 252.5 and 505 r, was given to mice in two groups of 20 each according to their body weight. Ten mice, in each group (5 gm and 10 gm), were not exposed to serve as control. Mice from each high and low dose irradiated groups and the unirradiated control were then challenged with SPV (10,000 SID 50) intracranially at the rate of 0.03 ml per mouse. Encephalitic symptoms and brains were observed at the end of 10 days for any gross lesions.

##### In Tissue Culture

The monolayers of chick and mouse embryo fibroblast in 4 oz. bottle were infected with SPV (10,000 SID 50) by inoculating 1 ml per bottle. After virus adsorption at 37°C for 1 hr 2 ml of maintenance medium was added and cultures were reincubated at 37°C for 140 hours. The virus was liberated by freezing and thawing and centrifugated at 3000 rpm. for 10 min. to remove coarse particles. Such four successive passages were given.

The virus from passaged material from animals and tissue cultures were inoculated i/d in lambs to observe any loss in virulence.

##### In Intact Animals

Macroscopic examination of brains of 4-day-old mice and rats inoculated intracranially in each passage, revealed no lesions.

Hyaluronidase is known to facilitate the spreading of virus, and testis are known to be rich in this enzyme (Monroe *et al.*, 1949; Sen, 1968). Male mice and rats of varying age groups, i.e., 20, 40 and 60 days, were, therefore, inoculated intratesticularly. Macroscopic examination revealed no change.

Passage of SPV in rabbits and guinea pig testis did not bring about any gross change.

The visual examination of the SPV inoculated CAM revealed thickening of the membrane which increased progressively on successive passages, such thickening was not found in the control membranes.

The passaged materials obtained from mice, rats, rabbits, guinea pigs and embryonated eggs were inoculated in lambs skin intradermally, neither they produce any gross lesion nor any rise of body temperature upto 10th day. On challenge with virulent SPV, no protection of the inoculated lambs was observed, showing that SPV apparently did not multiply in mice and rats brain, mice, rats, rabbits, guinea pig testis as well as on CAM of eggs.

Groups of mice were irradiated with 252.5 r and 505 r prior to inoculation of the SPV intracranially, observation revealed that irradiation of mice apparently did not have any effect on virus adapta-

tion. No symptoms of encephalitis or gross lesions in brain were found in irradiated mice.

#### Cultured Tissue Cells

Mouse and chick embryo fibroblast passaged SPV when inoculated in lamb skin, only mouse embryo passage was able to cause a certain visible reaction in lambs and infected animals died after 14 days with specific symptoms of sheep pox infection. While no reaction was observed with chick embryo passaged material.

On challenge with virulent SPV the lambs died after 10 days of post-infection.

The fact that attempts by workers to adapt and attenuate sheep pox virus, in heterologous intact hosts and their cultured tissue cells, have failed suggests that we may have inadequate information about the physico-biological properties of the virus itself. Our attempt to adapt SPV in suckling mice and rats and adult male mice, rats, rabbits, guinea pig and chick embryo, intracranially and intratesticularly, met with failure. Similar negative results have also been reported by Angeloff (1940), Sen (1968), Ortenzi (1954), and Ozcebe *et al.* (1958), while, Trotsenko (1961) had reported success in the adaptation of a chinese strain of sheep pox virus in chorio-allantoic membrane.

In the present studies, cultured tissue cells of mouse embryo could be infected by SPV. Passaged virus produce specific symptom of sheep pox disease in lambs preceded by death.

The authors are grateful to Dr. M. L. Dhar, Director, C.D.R.I., for providing facilities and to Dr. R. C. Pathak, Vety. College, Mathura, for the supply of virus.

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#### ON THE FREQUENCY OF OCCURRENCE OF PINNOTHERES SP. IN THE WINDOW-PANE OYSTER, PLACENTA PLACENTA (LINNE)

HORNELL AND SOUTHWELL<sup>1</sup> and Chhapgar<sup>2</sup> have described and figured *Pinnotheres placunae*. Silas and Alagaraswamy<sup>3</sup> have reviewed the available literature on the systematics, ecology, biology and ethology of the pea-crab of the genus *Pinnotheres* (Latreille) while giving an instance of parasitisation by the pea-crab *Pinnotheres* sp. on the back-water clam *Meretrix casta*. The species identification of the pea-crab presently reported is under scrutiny. The frequency of its occurrence in eighty forms of *Placenta placenta* collected from Kakinada Bay, on the east coast of India during December, 1973 is reported here. Based on the existing literature on the subject it can be said that this is the first report on *Pinnotheres* sp. in *Placenta placenta*.

A total of sixty-four out of the eighty window-pane oysters (80.0%) examined were infested with one or more pea-crabs. An analysis of the frequency of occurrence of the pea-crabs in the infested forms showed the following position:

	No.	%
Number of infested forms out of eighty examined	.. 64	80.0
Number of forms with single crab each	.. 63	79.0
Number of forms with two crabs each	.. 1	1.2

For sex-wise occurrence and stage of development of crabs, the window-pane oysters were specially examined. The examination has revealed that there were sixty-one females and four males as follows:

Female	No.
Stage I (Hard-shelled stage)	.. Nil
Stage II (Soft-shelled stage)	.. 1
Stage IV (Adult, Non-ovigerous)	.. 13
Stage V (Adult, Ovigerous)	.. 47
Total	.. 61

Male	No.
Stage I (Hard-shelled stage)	.. 1
Stage II (Soft-shelled stage)	.. 3
Total	.. 4

A further analysis of the single and multiple infestations by the pea-crabs in the sixty-four infested window-pane oysters examined shows the following:

Single infestation	No.
Total Number	.. 63
Female-stage I (Hard-shelled stage)	.. Nil

Female-stage II (Soft-shelled stage) ..	1
Female-stages IV and V (Adults) ..	59 (13 + 46)
Male-stage I (Hard-shelled stage) ..	Nil
Male-stage II (Soft-shelled stage) ..	3

**Multiple infestation**

Double infestation

Total number ..	1
Female-stages IV and V (Adults) ..	1 (0 + 1)
Male-stage I (Hard-shelled stage) ..	1

The present study has shown that the number of female crabs in *Placenta placenta* during December, 1973 is greater than that of the males and that a large number of the females are ovigerous. In most of the ovigerous females the egg mass is light to dark brown in colour. There is presently only a single instance of double infestation.

Christensen and Mc Dermott<sup>4</sup> have suggested that the 'deficiency' of males may have been due to a natural death of males after copulation and also that some of them may have fallen prey to predators while moving from one oyster to another in search of females. Christensen and Mc Dermott<sup>4</sup> and Silas and Alagarwamy<sup>3</sup> have also reported that each double infestation consisted of one crab of each sex.

Our grateful thanks are to the authorities of the Andhra University for providing facilities to carry out the present work. The Junior author takes this opportunity to sincerely thank the C.S.I.R. for the award of a Junior Research Fellowship.

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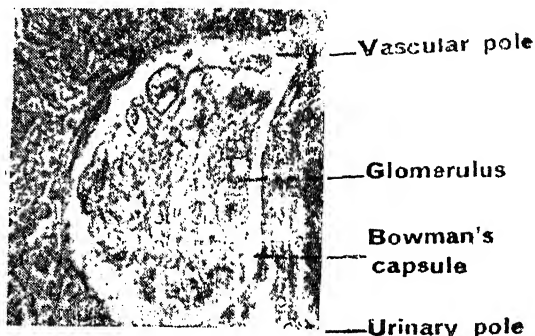
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#### A NOTE ON THE PRESENCE OF "GIANT GLOMERULI" IN A SISORID CAT FISH *GLYPTOTHORAX KASHMIRENSIS* HORA

A HISTOLOGICAL study of the kidney of *Glyptothorax kashmirensis* has revealed the presence of some well vascularized "Giant glomeruli". These are randomly distributed throughout the kidney and may occur in groups of two or more at a place.

The average size of a "Giant glomerulus" is  $148 \times 155$  micra compared to  $75 \times 66$  micra of the normal glomeruli. While the normal glomeruli are round or oval in shape the "giant" ones are

almond shaped. The "Giant glomerulus" (Phm. 1) is structurally similar to the normal glomerulus. The Bowman's capsule is lined with squamous epithelium which is also reflected on the glomerulus. The vascular and urinary poles are also present.



PHM. 1. Photomicrograph of a "Giant glomerulus" of *Glyptothorax kashmirensis*.

Large well vascularized glomeruli have been reported also in yellow bull head, *Ictalurus natalis*<sup>1</sup>. Presence of a small number of large sized glomeruli is considered to be a primitive character<sup>2</sup>. It may be that, but it appears more likely that fishes like *G. kashmirensis*, which inhabit clear hill stream waters have evolved "Giant glomeruli" in order to increase the filtration surface and thereby solved osmoregulatory problems faced by them.

It is also noteworthy that the occurrence of "Giant glomeruli" in a small fish like *G. kashmirensis*, hardly weighing 12 gm on an average, is a departure from the generalisation that size and number of glomeruli varies directly with the weight of the fish<sup>2</sup>.

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Govt. College Udhampur J. & K.,  
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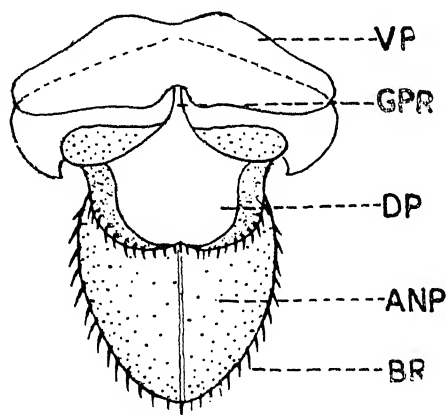
#### A STUDY ON THE FEMALE EXTERNAL GENITALIA OF *HIPPOBOSCA MACULATA* (LEACH)

THE present paper deals with the morphology of female external genitalia of *Hippobosca maculata* Leach, ectoparasite of cattle and horses. The work of Theodar (1953) on the structure of genitalia in Nycteribiidae is a valuable work for reference.

Mukerji and Dasgupta (1954) have also described the female genitalia of *Cyclopodia sykesi* (Nycteribiidae).

The flies were collected from cattle and horses at Indore. For external genitalia the abdomen is boiled in 10% KOH for transparency, material washed and neutralised with Acetic Acid. After dehydration, slides were mounted in Canada balsam. Temporary mounts in Berlese fluid gave better results.

The female external genitalia of *H. maculata* are reduced as in other Pupipara. These are represented by two genital plates, one dorsal (DP) and another ventral (VP) (Fig. 1). Both plates are hidden under



0.4 MM

FIG. 1. External genitalia of female *Hippobosca maculata* in ventral view ANP—anal plate; BR—bristles; DP—dorsal plate; GPR—gonopore; VP—ventral plate.

a large dorsally placed horse-shoe shaped analplate (ANP), beset with numerous setae. Thus the genital plates are not visible from dorsal side. They can be seen by removing the anal plate. The presence of genital plates is also reported by Theodar (1953) in *Nycteribiia*, *Penicillidia* and *Eucampsipoda* (Nycteribiidae).

The dorsal plate is larger than the ventral plate. The differentiation can be seen from the ventral view of the abdomen. Anteriorly the dorsal plate is convex while posteriorly notched or concave and bears numerous setae. The ventral plate is small and chitinated, divisible into two similar wing-shaped halves. Both the plates are connected by a thin pleural membrane and guard the female gonopore (GPR). The dorsal plate is not attached to the anal plate as reported by Theodar (1953) in *Eucampsipoda*.

I am highly thankful to Dr. Ravi Prakash, Head, Department of Life Sciences, University of Indore, and now Vice-Chancellor, University of Bhopal, for guiding and providing facilities during the present work.

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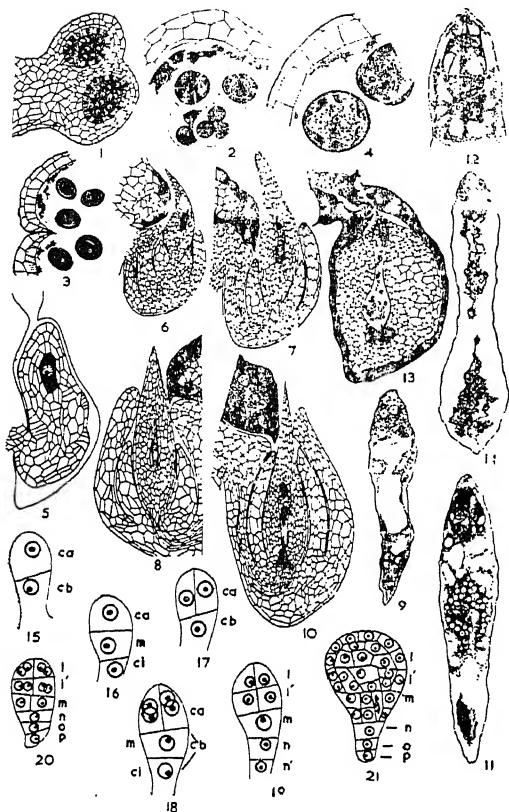
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#### SOME EMBRYOLOGICAL FEATURES OF *EUPHORBIA VERMICULATA* RAF.

SPECIES of the genus *Euphorbia*, besides displaying extreme morphological diversity, show features of utmost embryological interest. As far as is known, the occurrence of as many as four types of embryo sac development, namely, Polygonum, Peperomia, Allium and Fritillaria, seem to be an outstanding feature of *Euphorbia*<sup>1,3-6</sup>, since all other genera of the Euphorbiaceae investigated so far are reported to show one or the other of the types of embryo sac development. To date, our embryological knowledge of *Euphorbia* is restricted to very few species. The following contribution presents, from work in progress, the more important embryological features of *Euphorbia vermiculata* Raf.

The anther is tetrasporangiate. An anther lobe at microspore mother cell stage comprises the epidermis, endothecium, a single middle layer and the secretory tapetum (Fig. 1). The development of the anther wall corresponds to the dicotyledonous type<sup>2</sup>. The tapetal cells remain uninucleate throughout and start degenerating *in situ* about the time the microspore tetrads are formed (Fig. 2). The fibrous thickenings are laid down in the endothelial cells at the one-celled stage of the pollen grains (Fig. 3) and they remain feeble even after anthesis. The microspore mother cells undergo the regular meiotic divisions of the simultaneous type resulting mostly in tetrahedral tetrads (Fig. 2). The spheroidal tricolpate pollen grains are shed at the 2-celled stage (Fig. 4). The exine of the pollen grain is sharply differentiated into an outer thick sexine bearing minute but dense echinulations and a ridged nexine (Fig. 4).

The ovary is superior, tricarpeal, syncarpous and trilocular with a single anatropous, bitegmal and crassinucellar pendulous ovule in each locule borne on axile placentae. The integumentary



FIGS. 1-21. Fig. 1. T.s. Anther lobes at microspore mother cell stage showing a four-layered wall. Note 1-nucleated tapetal cells,  $\times 180$ . Fig. 2. T.s. A portion of the anther lobe to show epidermis, endothecium, degenerated middle layer, disorganising tapetum and tetrahedral tetrads,  $\times 180$ . Fig. 3. T.s. A portion of the anther lobe to show the feebly developed fibrous thickenings in endothelial cells at 1-celled stage of the pollen grains,  $\times 180$ . Fig. 4. T.s. A portion of the anther lobe showing 2-celled pollen grains. Note the fibrous thickenings remaining feeble even at the 2-celled stage of the pollen grains,  $\times 180$ . Fig. 5. L.s. Ovule primordium showing the initiation of the integumentary primordia. Note the megaspore mother cell in the nucellus,  $\times 300$ . Fig. 6. L.s. Anatroous ovule at megaspore mother cell stage. Note the outer integument outgrowing the inner, nucellar beak, deep seated megaspore mother cell and placental obturator,  $\times 180$ . Fig. 7. L.s. Ovule to show a linear tetrad of megaspores and growing placental obturator,  $\times 180$ . Fig. 8. L.s. Ovule showing 2-nucleate embryo sac and degenerated megaspores,  $\times 180$ . Fig. 9. Embryo sac with eight nuclei,  $\times 300$ . Fig. 10. L.s. Ovule showing organised embryo sac,  $\times 450$ . Fig. 11. Organised embryo sac. Note the starch grains,  $\times 450$ . Fig. 12. Micropylar part of the embryo sac showing egg apparatus and the two polar nuclei at the vicinity of the egg at fertilisation,  $\times 600$ . Fig. 13. L.s. Developing seed to show the globular embryo, disorganising nucellar beak, cellular

primordia appear at the megaspore mother cell stage (Fig. 5) and their further growth seems to be rather delayed. The inner integument is the first to differentiate (Fig. 5), but the outer subsequently outgrows the inner (Figs. 6, 7, 8). The nucellus is massive and projects far beyond the micropyle as a prominent nucellar beak (Figs. 6, 7, 8, 10). The placental obturator differentiates very early, but attains its maximum development at the 8-nucleate stage of embryo sac (Fig. 10) and shows signs of disorganisation after fertilisation (Fig. 13). At the chalazal region, below the embryo sac, a pad of tissue consisting of richly cytoplasmic and uninucleate cells is discernible at about 8-nucleate stage of the embryo sac (Fig. 10) and becomes more and more prominent in the ovule after fertilisation (Fig. 13).

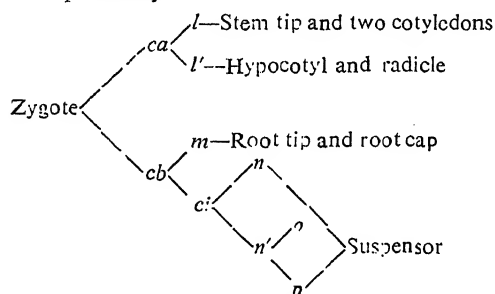
The megaspore mother cell, which is deep seated in the nucellus (Fig. 6) undergoes the customary meiotic divisions to develop a linear tetrad of megaspores (Fig. 7). The chalazal megaspore functions (Fig. 8) and its nucleus undergoes the three successive mitotic divisions leading to the formation of four nuclei at each of the proximal and distal ends of the embryo sac (Fig. 9). The eight nuclei organise in a manner distinctive to the polygonum type of development (Figs. 10, 11). The polar nuclei fuse in the vicinity of the egg (Fig. 12) and form the secondary nucleus. The three antipodal cells degenerate even before the fusion of the polar nuclei (Figs. 10, 11). The cytoplasm at the central region of the embryo sac is richly studded with starch grains (Fig. 11).

The development of the endosperm is Nuclear. Endosperm formation begins long before the first division of the zygote and at the 2-celled stage of the pro-embryo twenty free nuclei are noted in the embryo sac (Fig. 14). The nuclei of the developing endosperm show accumulation around the embryo and at the antipodal end, while they are relatively few at the sides. When the embryo is at globular stage, cell wall formation commences from the micropylar end and proceeds toward the antipodal end (Fig. 13).

The embryo development follows the *Euphorbia* variation of the *Onagrad* type. The division of the zygote occurs invariably after that of the primary endosperm nucleus. The first division is transverse engendering the terminal cell *ca* and basal cell *cb* (Figs. 14, 15). The basal cell then divides trans-

endosperm at the micropylar part of the embryo sac, well organised hypostase and the disorganising placental obturator,  $\times 180$ . Fig. 14. Embryo sac with 2-celled proembryo and 20 free endosperm nuclei,  $\times 300$ . Figs. 15-21. Different stages in the development of embryo,  $\times 450$ .

versely to form *m* and *ci* either before or after the vertical division of the terminal cell (Figs. 16, 17, 18). A vertical wall in *ca* results in two juxtaposed cells, which divide again by another longitudinal wall at right angles to the preceding one resulting in a quadrant (Fig. 18). Subsequently each cell of the quadrant divide transversely forming an octant resulting in two tiers *l* and *l'* of four cells each (Figs. 19, 20). The upper four cells of the octant ultimately give rise to the stem tip and the two cotyledons, while the lower tier of cells functions to form the hypocotyl and radicle. The cell *m*, derived from *cb*, produces the root tip and root cap, while *n*, *o* and *p*, derived from *ci*, constitute a three-celled suspensor (Fig. 21). The scheme given hereunder summarises the origin of the different parts of the embryo from the cells of the proembryo :



Work on other exotic species of *Euphorbia* is in progress.

Grateful appreciation is hereby expressed to Professor T. Sreeramulu, for his constant encouragement. Thanks are also due to Professor C. J. Hillson, Pennsylvania, for sending the material and to the Council of Scientific and Industrial Research, New Delhi, for the award of Junior Research Fellowship to one of us (Miss K. R. R. Devi).

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## IS THE EMBRYO SAC OF PODOSTEMACEAE BISPORIC?

IN SPITE OF repeated investigations and varied interpretations put forward, the exact nature of the embryo sac development in Podostemaceae requires a proper explanation. Recently, Battaglia<sup>1</sup> has reviewed the previous literature on the embryo sac of Podostemaceae. He classifies the embryo sac development in this family under the following types: the Dicraea type, the Podostemum type and the Apinagia type. These types of embryo sac development have been accepted without any reservation by all embryologists, who have investigated Podostemaceae, as a reduced bisporic type<sup>2-12</sup>.

Before discussing the types of embryo sac development in Podostemaceae, it is necessary to know the criterion employed in the classification of the types of embryo sacs in angiosperms. P. Maheshwari<sup>13</sup> classified the types of embryo sac development in angiosperms into the mono-, the bi-, and the tetrasporic types. Although these terminologies are in wide usage, there is confusion regarding their exact definition. Some authors regard merely the number of megaspores that contribute to the formation of the organised embryo sac as the criterion<sup>14-15</sup>, while others consider only the number of megaspore nuclei that participate in the embryo sac organisation<sup>16-17</sup>. To avoid this confusion, it appears appropriate at this stage to recall the original definition put forward by P. Maheshwari (1937, p. 360) which reads thus "... there is a general consensus of opinion about regarding the first four nuclei formed after the reduction divisions as equivalent to megaspore nuclei; the laying down of a wall separating them is a matter of secondary importance. Consequently, an embryo sac formed from the divisions of a single megaspore nucleus should be called *monosporic*; when two take part in its development, it is *bisporic*; and when all four contribute to it, it is *tetrasporic*".

If the above definition is carefully examined, then the development of the embryo sac in Podostemaceae needs a critical reconsideration. The Dicraea type\* and the Podostemum type must be considered as truly bisporic because in both cases two megaspore nuclei are involved in the subsequent divisions and therefore contribute nuclei to the organised embryo sac. The megaspore mother cell divides to form two dyad cells. The micropylar dyad cell degenerates while the chalazal dyad cell enlarges and divides to produce two megaspore nuclei which again divide to produce four nuclei which in turn take part in the organisation of the embryo sac. The difference between the Dicraea type and the Podostemum type lies in the organisation of the cells contributing to the mature embryo sac.

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On the other hand, in the Apinagia type of embryo sac development, the megaspore mother cell undergoes the I-meiotic division to produce a dyad of two cells. The micropylar dyad cell may or may not undergo the II-meiotic division but soon degenerates. The nucleus of the chalazal dyad cell undergoes the II-meiotic division to produce two megaspore nuclei which move apart to the poles of the embryo sac. The megaspore nucleus situated at the chalazal end degenerates, while the one situated at the micropylar end divides twice and contributes to all the four nuclei present in the organised embryo sac. Went<sup>11</sup> for the first time observed this type of development in *Oenone imthurnii* and *Mourera fluviatilis* and this was subsequently named as the Oenone type of embryo sac development<sup>1</sup>. Recently Battaglia<sup>1</sup> for nomenclatural reasons renamed this Oenone type as the Apinagia type. It has been fairly well established that in a majority of the so far embryologically investigated taxa of Podostemaceae there is the Apinagia type of embryo sac development.

The Apinagia type will have to be looked upon as *monosporic* because only one megaspore nucleus contributes to all the four nuclei present in the organised embryo sac. Instances of occasional division of the chalazal megaspore nucleus reported by Razi<sup>8</sup> needs confirmation. Whether the chalazal megaspore nucleus persists in the mature embryo sac or not, it is logical and reasonable to conclude that the Apinagia type of embryo sac development be treated as monosporic and tetranucleate.

I am deeply grateful to Dr. D. A. Govindappa for his inspiring guidance. I sincerely thank Dr. K. Subramanyam, former Director, Botanical Survey of India and Prof. V. Puri of Meerut University for scrutinising the manuscript.

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\* It is more appropriate to call it as *Polypleurum* type because the genus *Dicraea* is now *Polypleurum* (See Hall<sup>15</sup>).

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#### LATE BLIGHT OF POTATO *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY

LATE blight of potato hitherto found only in the Northern States of India has been recorded for the first time in Maharashtra State.

While carrying out survey for diseases in field crops round about Panchagani and Bhilar (Dist. Satara, Maharashtra), leaves of potato were found to be affected by a disease. On critical microscopic observations, these were invariably found to be infected by fungus *Phytophthora infestans* (Mont.) de Bary.

The disease usually starts from the leaf margins with the appearance of chlorotic patches which later on turn brown. Disease develops rapidly, with high humidity and fog, causing complete defoliation and rotting of tubers, leaving barren stems resulting in 50 to 70% loss in yield of tubers.

Fungus develops whitish haze, consisting of sporangiophores bearing sporangia in great number in favourable climatic conditions. Mycelium is inter- and intracellular, hyaline, non-septate, 6 to 10  $\mu$  broad, producing 2-4 sporangiophores bearing sporangia at the tip, which are lemon-shaped, colourless, measuring from 28 to 34  $\mu \times$  17 to 21  $\mu$ . Thick-walled hyaline oospores are also produced by the fungus.

Regional Wheat  
Rust Research  
Station,  
Mahabaleshwar,  
Dist.: Satara,  
Maharashtra State,  
September 27, 1973.

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V. V. SHINGTE.  
S. B. HUKERI.  
B. B. KHOT.  
S. B. LOKHANDE.

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## REVIEWS AND NOTICES OF BOOKS

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**Ecology: An Evolutionary Approach.** By J. Merriam. (Addison-Wesley Pub. Co., Reading, Massachusetts-01867), 1973. Pp. xiv + 493. Price \$17.25.

The science of ecology has definitely progressed beyond its natural history phase and can now boast of a substantial body of theoretical formulation. Such theoretical formulation ultimately derives either from thermodynamics or from the principle of natural selection. The thermodynamic approach finds its expression in the works of Odums, and is the approach generally emphasized in the Indian Universities. Emlen's book is a comprehensive presentation of the second approach based on the principle of natural selection. This evolutionary approach is based on the notion that all the characteristics of a living organism are constantly being moulded by natural selection so as to maximise its genetic fitness. We therefore expect all the ecological attributes such as the number of young produced in a litter, the size of prey taken, the amount of distance over which the young disperse and so on to confer maximal genetic fitness under the prevailing environmental conditions on the genotype concerned. The application of this idea may be illustrated by the classic studies of David Lack on clutch size in birds. He argued that the number of eggs laid by any bird species must be such as to ensure that the maximum number of young are successfully raised. If the clutch is too large, many young will be inadequately fed and die of starvation, if too small, the ability of parents to feed the young is not taken full advantage of. Since there is a certain variation in nature in the size of the clutch, we may expect the most frequent clutch size to correspond to that leading to the maximum number of successfully raised young. Lack's field studies showed that this expectation was in fact borne out in many cases.

Lack derived much inspiration from the conceptual advances brought about by the work of Lotka, Haldane, Fisher and Gause, who had pioneered the application of rigorous mathematical reasoning to biological problems. Present-day evolutionary ecology is a happy blend of Lack's brand of informal theorisation and Haldane's brand of mathematical modelling, of Gause's brand of population experiments and of classical natural history. The discipline has rapidly matured during the last twenty years, and by now supplies the

paradigm followed by a large school of ecologists. Emlen's book is a very useful and competent review of the field and would serve the need earlier fulfilled by Slobodkin's charming little book which has now become rather outdated. MADHAV GADGIL.

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**Genetics and Society.** Edited by Jack B. Bresler. (Addison-Wesley Publishing Co., Massachusetts), 1973. Pp. xv + 280. Price \$5.20.

This volume presents a variety of essays and attempts to introduce students to the newly emerging, rapidly developing field of 'Socio-genetics'.

The eugenic contributions of H. J. Muller are significant and permeate all discussions which relate genetics to Society. The influences which have acted on Muller as well as the chronological progression of his thinking on Science and culture are reviewed by Allen.

The section on 'Basic genetic patterns' contains papers which explore relationship: individual, familial and social through the simpler genetic patterns of dominance, recessiveness, and twin development. The reports indicate that social implications are derived from genetic patterns and genetic implications from social contacts.

The topic of 'Human chromosomes and antisocial behaviour' examines the "Human chromosome abnormalities as related to physical and mental dysfunction".

Some of the deepest social and political controversies on radiation, intelligence, prenatal influences and postnatal influences constitute the subject-matter of the section on 'Genetics and early human development'.

Ethnic groups frequently have characteristics which are largely genetic in origin. This aspect is well brought out in the papers, 'Gene frequencies in Jews'; 'Welshness and fertility' and the 'Behavioral differences between Chinese-American and European-American New borns'.

Characteristics of ethnic group and the outcome of matings between ethnic and racial stock are depicted in the papers: 'The Founder effect and deleterious genes', 'Genetic aspects of plantation slavery' and 'Outcrossings in Caucasians and fetal loss'.

'Genetic counselling as an integral part of medical care' and a fascinating review of the different systems of laws and different interpretations of



genetic relatedness as a basis for prohibition of inbreeding, adopted in the United States, highlight the aspect of "Human restructuring of his own species".

M. SIRSI.

**Annual Review of Biophysics and Bioengineering** (Vol. 2). (Annual Reviews, Inc., Palo Alto, California, U.S.A.), 1973. Pp. vii + 333. Price \$ 12.00 in U.S.A.; \$ 12.50 elsewhere.

This volume also keeps the same high standard as the earlier Volume 1. Since a number of different subjects are considered in the volume, the contents are best described by the following titles and authors of the various articles: Aaron Katchalsky (Obituary); Frequency Dynamics of Peripheral Vascular Blood Flow, E. O. Attinger and R. M. Attinger; Electric and Magnetic Field of the Heart, David B. Geselowitz; Interpretation of Some Microelectrode Measurements of Electrical Properties of Cells, A. Peskoff and R. S. Eisenberg; Clustering, J. A. Hartigan; Technology of Multiphasic Patient Screening, Morris F. Collen and Joseph F. Terdiman; Optimization of the Mammalian Respiratory Gas Transport System, Fred S. Grodins and Stanley M. Yamashiro; Primary Processes in Bacterial Photosynthesis, Roderick K. Clayton; Toward Direct Brain-Computer Communication, Jacques J. Vidal; Biophysics of Flagellar Motility, J. J. Blum and J. Lubliner; Long-Range Physical Forces in the Biological Milieu, V. Adrian Parsegian; Structure and Symmetry of Oligomeric Enzymes, B. W. Matthews and S. A. Bernhard.

The volume opens with a short obituary of Prof. Aaron Katchalsky who was unfortunately killed by terrorists' bullets at the Tel Aviv airport. He was certainly one of the world's outstanding biophysicists. It is very tragic that Katchalsky's death occurred at the height of his abilities and influence. Throughout his scientific life, his primary interest was to understand the molecular mechanisms of life processes. Active transport in biological membranes is a field that received much elucidation as a result of Katchalsky's studies. He was

also greatly interested in brain functions, with special reference to the application of biophysical chemistry for its understanding.

Perhaps the most interesting of the articles in this volume is the one about Direct Brain-Computer Communication by J. J. Vidal. He raises the question whether observable electrical brain signals can be put to work as carriers of information in man-computer communication. The author believes that the purposeful use of such a communication is just around the corner.

Another interesting article is on the Structure and Symmetry of Oligomeric Enzymes by B. W. Matthews and S. A. Bernhard. They discuss in particular the modes of association of protomers in such systems, including the possible point group symmetries that they may have. They describe techniques like electron microscopy and X-ray crystallography which provide useful information regarding this feature and they list a large number of examples in which the required information is available. The chemistry of association and dissociation of such enzymes and their ligand binding are particularly discussed. They have also summarized the structural data currently available from X-ray crystallographic studies of a number of oligomeric proteins.

The volume contains very useful information for biophysicists and bioengineers and should find a place in every library devoted to these subjects.

G. N. RAMACHANDRAN.

#### Books Received

*WHO Technical Note No. 126—Comparison Between Pan and Lake Evaporation.* By C. E. Hounam; Pp. iv + 52. Price not given; *Technical Note No. 129—Energy Fluxes Over Polar Surfaces.* Edited by Sverre Orvig; Pp. iv + 299. Price not given.

*Acta Botanica Indica* (Vol. I, Nos. 1 and 2), 1973. (Society for Advancement of Botany, Department of Botany, Meerut College, Meerut-250001), Pp. 75. Annual Subscription: Individual Rs. 25.00 or £ 2.50 or \$ 7.00; Institution: Rs. 40.00; £ 4.00; \$ 10.00.

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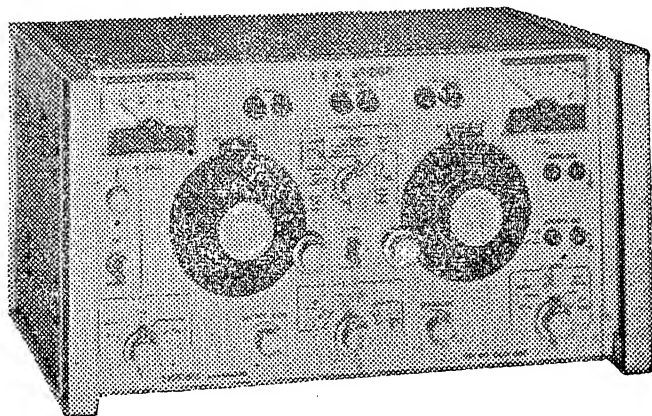
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## ABSTRACT

Rare earth complexes of kojic acid of the general composition  $[\text{Ln}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$  (where Ln represents  $\text{Dy}^{+3}$ ,  $\text{Ho}^{+3}$  and  $\text{Y}^{+3}$ ) have been studied. These complexes have been isolated and characterised by analyses, conductometric, amperometric, magnetic and I.R. spectral studies. Composition and I.R. spectra of the complexes show that kojic acid is acting as a bidentate ligand and the two water molecules are also present in the coordination sphere.

## EXPERIMENTAL

*Materials and Methods*

THE measurement of conductance and *i.e.*, spectra of the samples were made as reported earlier<sup>1</sup>. Kojic acid was of BDH (AR) quality. Solvents were reagent grade and were purified and dried before use.

A Toshniwal manual polarograph (CLO-2) with Pye scalamp galvanometer in the external circuit, was employed for carrying out amperometric titrations. The polarographic cell was kept immersed in a water thermostat maintained at  $30 \pm 0.1^\circ\text{C}$ .

susceptibilities were determined by Gouy's method, using copper sulphate pentahydrate as a calibrant. The rare earths (Dy, Ho and Y) were estimated as their oxides by direct combustion of the complexes. To make an actual estimate of water molecules bound per chelate, larger quantities of the compounds (0.4 g) were heated around  $120^\circ$  to constant weight. The percentage loss in weight was assumed to correspond to the number of water molecules. The microanalyses for carbon and hydrogen were carried out at the I.I.T., Kanpur. The analytical data are given in Table I, other physical properties are summarised in Table II.

TABLE I  
*Analytical data*

Complex		Carbon (%)	Hydrogen (%)	Metal (%)	Water (%)
$[\text{Dy}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	Calcd.:	34.78	3.05	26.14	5.79
	Found:	34.76	3.01	26.21	5.35
$[\text{Ho}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	Calcd.:	34.64	3.04	26.43	5.76
	Found:	34.20	3.05	26.33	5.80
$[\text{Y}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	Calcd.:	39.43	3.47	16.22	6.57
	Found:	39.49	3.09	16.19	6.10

TABLE II  
*Physical properties*

Complex	Colour	Yield (%)	Dec. temp.	Molar Conductance $\Omega - 1\text{ cm}^2\text{ mole}^{-1}$ ( $10^{-3}\text{ M}$ Nitromethane) <sup>5,6</sup>
$[\text{Dy}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	Cream	~50	260-290	23 (non-electrolyte)
$[\text{Ho}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	White	~60	270-295	29 do.
$[\text{Y}(\text{C}_6\text{H}_5\text{O}_4)_3(\text{H}_2\text{O})_2]$	Yellow	~65	250-295	25 do.

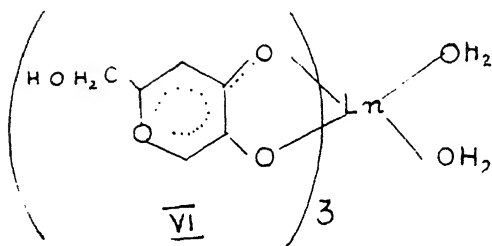
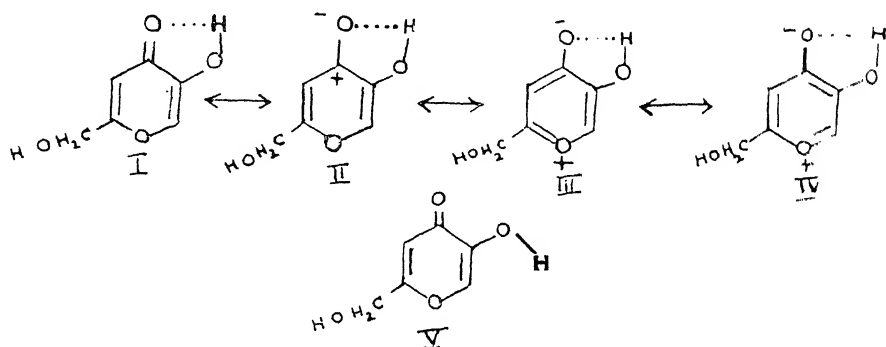
Fischer capillary with a drop time of 3.4 sec was used for the dropping mercury electrode. Purified hydrogen was used for deaeration. Magnetic

*Preparation of the Complexes.*—A general method employed for the preparation of rare earth complexes of kojic acid is described here.

Aqueous solutions of kojic acid (0.02 M) and lanthanide acetate (0.02 M) in the molar ratio 4 : 1 were mixed. The solutions were kept stirred by scratching the wall of the container; crystalline precipitates began to form in case of  $\text{Ho}^{+3}$ , whereas in the case of  $\text{Dy}^{+3}$  and  $\text{Y}^{+3}$  the precipitates were obtained after the concentration of the reaction mixture on a water-bath. The reaction mixtures, after keeping for about 2 hours at room temperature, were filtered and washed successively with water, ethanol and ether. The compounds were dried in vacuum.

ion has often been observed<sup>2</sup>. The magnetic moments observed for the kojates of dysprosium and holmium are 10.44 B.M. and 10.15 B.M. respectively which are in fair agreement with those reported for typical lanthanide sulphates<sup>3</sup>.

**Infrared Studies.**—The assignments of various bands of kojic acid and its complexes are presented in Table III. The presence of several absorption peaks in the region  $2400\text{--}3000\text{ cm}^{-1}$  in all the rare earth complexes show that hydrogen bonding is present in these complexes. Murakami *et al.*<sup>1</sup> have also observed the presence of hydrogen bonding in trans-



### Results and Discussion

Analytical results show 1:3 metal to ligand stoichiometry in all cases. All the rare earth complexes behave as non-electrolytes as revealed by their molar conductance measurements in nitromethane (Table II).

**Amperometric Titrations.**—Amperometric titrations, both direct and reverse, were carried out using 2 M potassium nitrate as supporting electrolyte and 0.2% gelatine as the maximum suppressor, at an applied potential of 1.2 V (metal) and 0.95 V (ligand) respectively. The results of these titrations also prove the existence of 1:3 complex in all these systems.

**Magnetic Measurements.**—The  $f$  electrons of the lanthanide ions are shielded from the perturbing effect of ligand by the outerlying  $s$  and  $p$  electrons. Therefore, a behaviour similar to the free gaseous

tion metal complexes and have proposed the following four resonating structures (I–IV) for kojic acid. The absorption bands at  $1680\text{ cm}^{-1}$  and  $1620\text{ cm}^{-1}$  in the spectra of the kojic acid have been assigned to the non-hydrogen bonded (structure V) and hydrogen-bonded (structure IV) carbonyl hydrogen modes, respectively. When kojic acid forms a complex with a rare earth ion (as is illustrated by structure VI), the former mode disappears and the latter shifts to lower frequencies.

The other significant bands, appearing at  $1585\text{ cm}^{-1}$  and  $1575\text{ cm}^{-1}$  in the free kojic acid which are due to stretching modes of  $\text{C}=\text{C}$  vibrations, also shift to lower frequencies through the formation of metal coordinate bonds. Thus, the shifts of both  $\text{C}=\text{O}$  and  $\text{C}=\text{C}$  bands to lower frequencies in the spectra of all the rare earth complexes seem to indicate that the contribution of resonance structures II, III and IV, in which a hydrogen atom is replaced by a metal ion, becomes greater as metal coordinate bonds are formed. The resonance interaction involving structures II, III and IV tends to delocalize olefinic and carbonyl  $\pi$  electrons over both  $\gamma$ -pyrone and chelate rings, thereby decreasing the double bond character of olefinic and carbonyl bonds.

Further evidence for the presence of a metal-oxygen bond in the complexes may be considered due to the disappearance of stretching and deforma-

TABLE III

*Significant peaks in the infrared spectra of lanthanide kojates (cm<sup>-1</sup>)*

Kojic Acid	Dy (III)-Kojate	Ho (III)-Kojate	Y (III)-Kojate	Assignment
3715-3725 (sb)	..	..	..	$\nu$ (OH) of Phenol.
..	3300-3400 (sb)	3300-3360 (sb)	3245-3445 (sb)	$\nu$ (OH) of H <sub>2</sub> O.
..	3,100 (m sh) 2,930 (w) 2,470 (w)	3,100 (m sh) 2,940 (w) 2,480 (w)	3,150 (m sh) 3,080 (w sh) 2,490 (w)	$\nu$ (OH) H-bonded.
1,680 (s)	1,600-1,620 (s)	1615-1630 (s)	1,620-1635 (s)	$\nu$ (C=O) of Ketone.
1,620 (s)	1,570-1590 (sb) <sup>a</sup>	1570-1595 (sb) <sup>a</sup>	1580-1600 (sb) <sup>a</sup>	
1,585 (s)	1,530 (w)	1,515-1,540 (w)	1,525-1,550 (b)	$\nu$ (C=C)
1,575 (m sh)	1,520 (w) 1,510 (w)	1,480 (s)	1,475 (w)	
1,381 (m)	..	..	..	$\delta$ (OH) of Phenol.
1,340 (s)				
..	1,025 (m) 980 (m)	1,000 (w) 970 (m)	970 (m) 945 (m)	$\delta$ r (H <sub>2</sub> O) Coordinated water.
860 (s)	860 (s)	885 (s)	885 (s)	
770 (s)	800 (s)	825 (s)	820 (s)	$\nu$ (C-H) out of plane.
..	750 (w)	775 (w)	775 (w sh)	$\delta$ w (H <sub>2</sub> O) Coordinated water.
..	390 (m)	390 (m)	400 (m)	$\nu$ (M-O)

s, strong; w, weak; Sh, shoulder; b, broad;  $\delta$ r, rocking;  $\delta$ w, wagging.<sup>a</sup> broadness of the band may be due to overlapping of  $\delta$  (H<sub>2</sub>O) of coordinated water.

tion OH of phenolic group on complexation. A weak absorption band around 400 cm<sup>-1</sup> has also been observed in the spectra of all complexes which may be due to M-(o) stretching vibrations.

## ACKNOWLEDGEMENT

Our thanks are due to Dr. W. U. Malik, Prof. and Head, Chemistry Department, Roorkee University, Roorkee, for his keen interest and valuable suggestions during the course of this work.

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## SUITABILITY OF EXPERIMENTAL DIETS FOR EARTHWORM CULTURE

J. V. BHAT

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SINCE Darwin focussed the attention of the scientific world on the earthworms through his famous book *Formations of Vegetable Mould through the Action of Worms, with Observations on Their Habits*<sup>1</sup> a good many investigators have carried out research work on one or the other aspect of these interesting invertebrates which reside in practically all soils of the globe. For over a decade from late forties the author, and his colleagues, had contributed to earthworm literature by furnishing information not only on the microflora associated with their alimentary canal<sup>2</sup> and the role they have in agriculture<sup>3</sup> with particular reference to the alteration they bring about on the microflora of soils<sup>4</sup>, but also by indicating the manner in which the worms bring about azotobacterization of soils<sup>5</sup>. However, no systematic efforts were made during the first decade of work to ascertain which diet(s) would suit most the requirements of their growth and reproduction or from the point of view of dissemination of *Azotobacter* which was shown to be associated with their alimentary canal as well as their castings. The purpose of this communication is to present the results of investigations carried out by the author during the second decade but withheld from publication as the author had not during the time in his collection more than one variety of earthworms for experimentation. Since then experiments upon other varieties have been conducted and confirmatory evidence obtained on the species studied earlier.

In the earlier studies<sup>6</sup> the practice adopted for culturing of the earthworms was on *ad hoc* basis of blending of soil with vegetable wastes including weeds, and animal waste (compost, mainly animal dung) in the proportion 8 : 2 : 2. Sometimes straw was also used. No systematic efforts were made to establish the superiority or otherwise of any one item over the other, or advantages to be derived by mixing of different dietary materials ascertained. In the experiments reported below a slightly alkaline soil was first chosen as the preliminary experiments revealed sudden drops in pH in the experimental diet in which the earthworms were reared. Secondly, since at the time, use of ammonium sulphate was commended as a fertilizer, a set of experiments were also carried out by incorporation thereof in soil, although it was obvious that earthworms fed upon organic matter and

would not make use of the mineral for their growth.

The experimental diets were made by mixing weighed quantities of red soil of pH 8.00 kept in mudpots (commonly used for growing garden plants) and incorporating therein on 10 : 1 dry weight basis cow dung, kitchen waste, green (leaf) manure, straw and a mixture of all the four above, and including ammonium sulphate. Ammonium sulphate was incorporated at the level recommended in soil as fertilizer. After blending, adult earthworms, in numbers specified in Table I, were introduced in the experimental diets and stabilization of conditions allowed to continue for 4 to 5 days, (but never over a week), and then, every two weeks, the earthworm populations were counted by hand picking. Microbiological analysis was limited to the use of modified Ashby's agar on which very accurate counts of *Azotobacter* (referred to as black colour colonies) could be made and which also permitted the growth of some other bacteria referred to in tables as colourless colonies. The significance of the latter remains to be ascertained. The microbiological analysis was not carried out after 4 weeks whereas earthworm populations were estimated upto the end of 7 weeks. The results of growth experiments in the experimental diets are presented in Table I.

From a glance at the table, it would be seen that the worms found the experimental diets 3, 4, 5, 6 and 7 as satisfactory as within 14 days adequate increase in progeny had taken place. Twenty-eight days utilization of the diet clearly showed that the worms perished in the presence of ammonium sulphate, (diet 2) whereas in others they multiplied reasonably well. Reproduction in the cow dung diet, green manure diet, and the straw diet was conspicuous. The parent progeny ratio, in fact, showed green manure and cow dung as superior to others, the ratio respectively for cow dung, kitchen waste, green manure and straw being 1 : 6, 1 : 3, 1 : 7 and 1 : 5.5. Closer examination of the results however shows that in the cow dung no cocoons were encountered, though 1 : 6 parent-progeny ratio was registered, whereas in others, cocoons were seen. Does this mean that cattle dung, being superior as will be clear from Table II permitted hatching expeditiously of the eggs? The answer seems to be yes from the fact that at no time the

TABLE I  
Earthworm population in soils

Sl. No.	Experimental Diets	Initial	After 14 days			After 28 days			After 47 days		
			A	Y	C	A	Y	C	A	Y	C
1.	Control Soil (CS)	6	6	..	..	6	..	..	4	16	..
2.	CS + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	8	8	1	..	..	..	..	..	..	..
3.	CS + Cow dung	10	10	2	..	10	61	..	12	126	..
4.	CS + Kitchen Waste	8	8	6	..	9	21	1	9	38	..
5.	CS + Green Manure	8	8	14	2	8	53	5	11	110	..
6.	CS + Straw	14	14	4	..	15	72	4	15	10	..
7.	CS + 2-6	16	16	9	..	16	37	..	16	38	..

A = Adult worms;

Y = Young ones;

C = Cocoons;

cocoons were encountered within 47 days in cow dung as well as in the green manure though in the latter they were there earlier; the young ones also attained maturity quickly and the population doubled itself. Straw feed, on the other hand, was unsuitable as the young worms died. Kitchen waste proved to be quite suitable whereas the mixture clearly was not so. The parent progeny ratio at this stage changed to 1:13, 1:5, 1:14, and 1:1 respectively. In the mixed diet, the ratio was at both stages 1:2, with approximation. Lack of food appears to have resulted in the death of two adults from the control though they seemed to have propagated to an extent.

A conclusion of greatest significance drawn from this experiment is that ammonium sulphate is highly detrimental to earthworm population and should never be used where earthworm culture is desired. In fact Edwards and Loft<sup>7</sup> conclude in their recent book that "There is good evidence that sulphate of ammonia is antagonistic to earthworm populations".

Results presented in Table II bring out clearly, the extent to which the experimental diets of the worms can effect the nature of the bacterial flora associated with them. Evidentially, only 3 of the 6 diets tried were observed to be suitable from the point of view of promoting azotobacterial growth, the population of which is important from the view-point of biological nitrogen fixation. Of these, straw seemed to be the medium of choice for their propagation. That addition of straw in soil did not adversely affect the overall nitrogen status, though brought about losses in nitrate nitrogen, was shown long ago by Murray<sup>8</sup>. Desai<sup>9</sup> had even

TABLE II  
Bacterial counts/g of soil containing exptl. diets on modified Ashby's agar\*

Experimental Diets	Initial**		After 14 days		After 23 days	
	B	C	B	C	B	C
1	..	700	..	1250	..	1700
2	..	500	..	900	..	1250
3	1850	500	2000	750	2150	1000
4	1150	400	1300	500	1400	500
5	..	1350	..	1700	..	2400
6	2550	450	3500	800	3700	900
7	..	450	..	1500	..	2450

\* Described in ref. 6.

\*\* After 5 days of stabilization on the introduction of the worms.

B = Black (*Azotobacter*) colonies.

C = Colourless colonies.

demonstrated nitrogen fixation under favourable conditions in soil in the presence of straw or organic matter. Likewise, Palacios and Bhat<sup>10</sup> also concluded from their studies on the effect of cellulose on the nitrogen status of soil exposed to different conditions of light and humidity that the presence of cellulose contributed favourably to its nitrogen status and that even after exposure of the soil to direct sunlight for 60 days in its dry state, the *Azotobacter* continued to live in soil. In other



words, availability of suitable carbon source must have been responsible for the *Azotobacter* to flourish in straw containing soil, the earthworms no doubt contributing, in their singular way, to its population. It may however be recalled, that in the presence of straw alone, earthworms could not reproduce beyond 30 odd days and, what is worse, young ones could not thrive much longer for reasons unascertained so far.

Next to the straw diet, cow dung and kitchen waste, in that order, appeared to be suitable for the *Azotobacter* to flourish. Surprisingly, the green manure, which proved to be superior for the rapid reproduction of the worms, failed to support the growth of *Azotobacter*. This, in fact, was the reason for considering above cow dung as superior to green manure for the rearing of the earthworms. The possibility that, if the experiments were continued beyond the period arbitrarily fixed, the earthworms population in cow dung would perhaps have exceeded that encountered in green manure, was indirectly evidenced from the observation that at no time cocoons were met with in the cow dung culture, whereas in the green manure culture, they were present at both the times of examination. Besides, the worms growing in cow dung appeared less immature and more alert; also, elicited better response than those reared in green manure to stimuli, e.g., touch; it is also interesting to note that the total number of bacteria growing on Ashby's medium recorded a higher count for the cow dung soil than did the kitchen waste soil, not to point out the relatively steady increase encountered in the *Azotobacter* count in the former.

That cow dung serves adequately the purpose of earthworms cultivation has been pointed out by Edwards and Lofty<sup>7</sup>. It was of interest to ascertain why the earthworm populations tended to dwindle or disappear in some of the diets. Determination of pH of the diets was considered in this context of holding the clue. Accordingly, pH at two stages, indicated in Table III, was measured with glass electrode. The results revealed the conspicuous and gradual fall in the ammonium sulphate soil and to a lesser extent in the soil containing the mixed diet. Wherever earthworms flourished, there was a tendency for the pH to rise after an initial fall, except in ammonium sulphate soil wherein the pH continued to fall and earthworm got killed at about pH 5.0. Kitchen waste also, at initial stage, lowered the pH and in all probability the rising earthworm population tended to counter such changes. In the mixed diet the ammonium sulphate, on the other hand, had its drastic effect, as even

TABLE III

pH of soils containing experimental diets and worms

Experimental Diets	Initial	After 14 days	After 28 days	After 42 days
1	8.0	8.15	8.0	7.85
2	6.2	5.3	4.9	4.55
3	8.15	7.9	7.9	8.00
4	7.8	7.4	7.35	7.5
5	7.9	7.8	7.8	7.95
6	8.0	7.9	7.9	7.95
7	6.55	7.1	5.85	5.3

after 47 days, there was no indication of any young worm attaining adulthood as witnessed with other diets.

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## OCCURRENCE OF TRANSLOCATORY NUCLEAR MOVEMENTS IN THE VEGETATIVE CELLS OF ANGIOSPERMS ALONG WITH A DISCUSSION ON THEIR CAUSATION AND MORPHOGENETIC SIGNIFICANCE

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### ABSTRACT

Occurrence of translocatory nuclear movements in the foot cells of filiform hooked hair of *Clitoria ternatea* L. and 2-armed hair of *Chrysanthemum indicum* L., during the ontogeny of these trichomes is demonstrated. The larger the cells, the more conspicuous is the nuclear movement observed. The movements are morphogenetically significant since they lead to cell divisions at a prescribed site, thereby contributing to development of the trichome conforming to its design. Nuclear movement being directional is interpreted to be a chemotropic response. The chemotropic factor is supposed to emanate from the site of prospective division and it is clearly indicated to be concerned with only the nuclear movement and not cell division. The events pertaining to the causation of nuclear movements and the consequences thereby are shown to occur in four major steps.

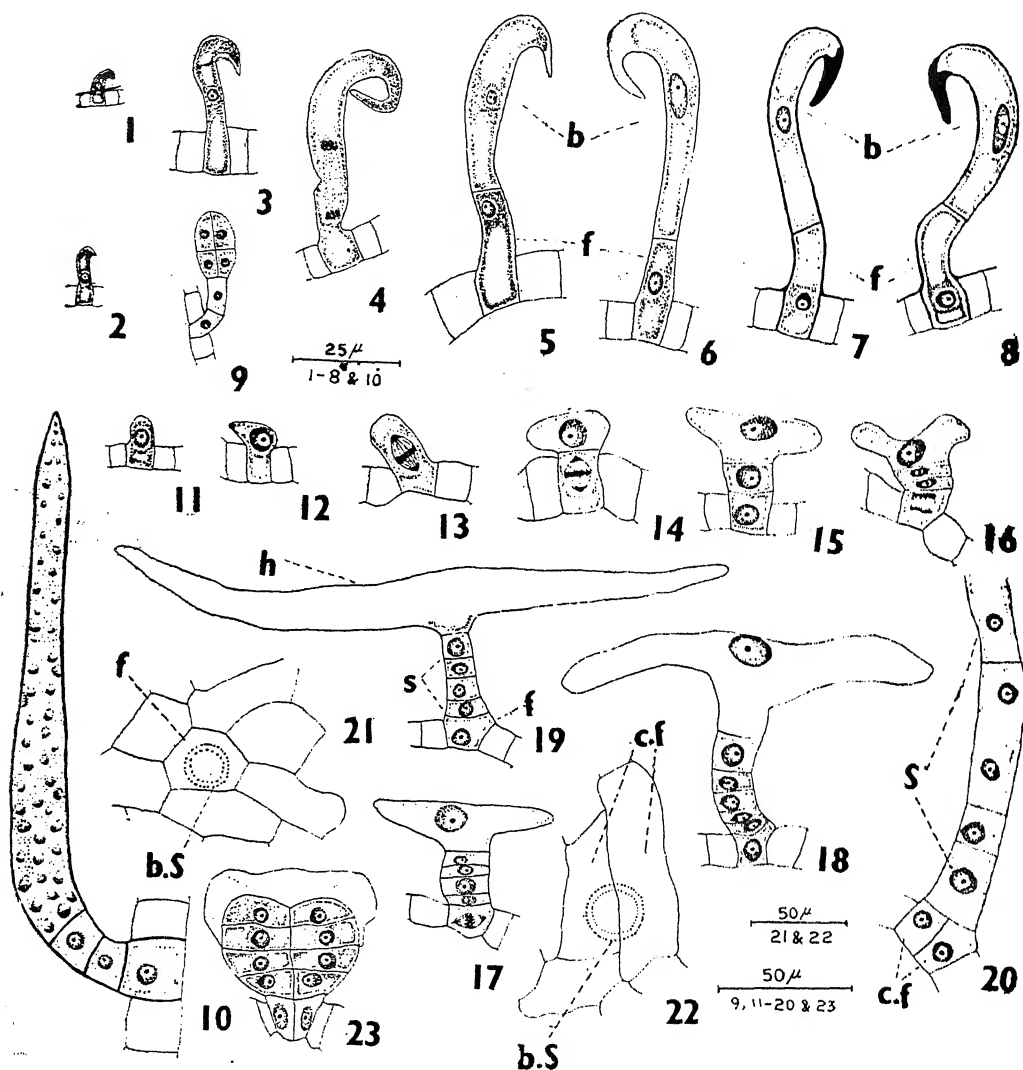
RECENTLY, Ramayya and Prabhakar<sup>7</sup> presented evidence on the occurrence of autonomous intracellular translocatory nuclear movements during the development of unicellular stigmatic hairs in *Helianthus annuus*, and *Chrysanthemum carinatum* and of the unicellular filamentous hairs in *Lagascea mollis*. In the angiosperms although interphase nuclear movement of polar nuclei preceding the secondary nucleus formation is universal, the evidence presented by these authors is important in that it was concerned with vegetative nucleus and was based on calibration of specific parameters<sup>7</sup>. The findings are significant since, though much understanding has been gained regarding varied other intracellular movements of cellular components,<sup>3,9-10</sup> there is little information concerning either the translocatory movements of interphase nucleus occurring in vegetative cells, or about their likely functional importance. In this paper, unlike with reference to unicellular trichomes, dealt with by Ramayya and Prabhakar<sup>7</sup>, evidence is presented on occurrence of interphase nuclear movements during the ontogeny of multicellular trichomes, the filiform hooked hair of *Clitoria ternatea* L. and 2-armed hair of *Chrysanthemum indicum* L. Further, comparing these movements with similar other situations an attempt is made to postulate on the likely factors involved in such movements and their morphogenetic significance during trichogenesis. Microtome slides prepared were stained with Ehrlich's haematoxylin<sup>2</sup>, and the epidermal peels with aniline blue in lactophenol<sup>8</sup>.

*Clitoria ternatea*.—Filiform hooked hairs (Fig. 8) occur on the stem, leaf, calyx, corolla and ovary of the species besides two others, the macroform conical hair (Fig. 10) and capitate glandular hair

(Fig. 9). The filiform hooked hair, ontogeny of which is concerned here, is uniseriate, 2-celled, curved into a hook at the distal end, slightly thick-walled and stiff (Fig. 8). Its basal cell which represents the foot of the trichome, is peculiar in that it is conspicuously projected far above the epidermis, whereas the upper cell forms the body of the trichome (Fig. 8). The trichome develops from a single protoderm initial which is recognisable from those of the other associate trichomes due to its smaller size and the curved tip (Figs. 1, 2), a condition leading to the hooked form of the hair at its maturity. At this stage the nucleus lies at nearly the middle of the initial and nearly at the same level as the protoderm (Fig. 1), but soon as the initial starts elongating, the nucleus also moves upwards appearing far above the protoderm level (Fig. 3). Now, the initial undergoes a transverse division giving rise to two cells (Fig. 4), the lower of which matures into the foot, while the upper one into the body (Fig. 5). In the latter the nucleus gradually moves upwards, whereas in the former it starts moving downwards (Fig. 6). At maturity the nucleus in the body cell usually comes to lie just below the hook-bend, whereas in the foot-cell it comes back to the position from where it had moved in the initial stage of the trichome (Figs. 6-8). It may be noted that while the nucleus is still on move in either of the cells of the trichome, the walls become secondarily thickened (Figs. 7, 8), the movement of the nucleus thus least affecting the process of sclerification. It is apparent from the trichome ontogeny that the nucleus of the trichome initial makes a to and fro movement, in which the forward move is obviously directed by the site

of the prospective cell division, whereas the rear move is a blind one.

*Chrysanthemum indicum*.—This bears two trichome types, 2-armed hair (Fig. 19) and biserial vesicular



FIGS. 1-23. Figs. 1-10. *Clitoria-ternatea* L. Figs. 1-8. Stages of development of filiform hooked hair. Figs. 1-6. From l.s. calyx (abaxial), Figs. 7 and 8. From l.s. bract (abaxial). Figs. 1-3. Show the nucleus moving upwards from trichome base before division. Fig. 4. Nucleus in division. Figs. 5-8. Nucleus making backward movement to the base of the foot cell after division. Figs. 9, 10. Capitate glandular and macroform conical hair respectively from a bract margin. Figs. 11-23. *Chrysanthemum indicum* L. Figs. 11-18. Stages of development of 2-armed hair. Figs. 11 and 12. Nucleus moving forwards before division. Fig. 13. First division of the nucleus. Figs. 14-16. Nucleus of the basal cell dividing antichinally to give rise to compound foot after contributing derivative to stalk formation. Fig. 18. 2-armed hair showing the nucleus of head cell near the outer wall due to the movement of the nucleus from the base (compare with Figs. 14 and 15). Fig. 19. Mature 2-armed hair from l.s. leaf (abaxial). Fig. 21. Surface view of foot cell of 2-armed hair after breakage of the trichome at the base of its stalk (leaf abaxial). Figs. 20 and 22. Compound foot of 2-armed hair in l.s. and surface view respectively (from leaf abaxial). Fig. 23. Biserial vesicular glandular hair (from leaf abaxial). (Figs. 13, 15-19. taken from Ramayya, 1969). (b = body; b.s. = base of the trichome stalk; c.f. = compound foot; f = foot; h = head; s = stalk).

glandular hair (Fig. 23) of which the former, the ontogeny of which is concerned, here, is borne on leaf, stem, peduncle, phyllaries, corolla and ovary of the plant. The 2-armed hair is distinguishable into foot, stalk and head portions (Fig. 19). The foot may be unicellular or simple (Figs. 19, 21), or compound being usually 2- or 3-celled (Figs. 20, 22). The trichome develops from single protoderm initial (Figs. 11, 12) and as described in detail earlier by Ramayya<sup>6</sup>, a given initial usually divides thrice transversely giving rise to stalk and head (Figs. 13-17) subsequent to which it may as such mature into a simple foot (Figs. 18, 19, 21) or again divide anticlinally once or twice (Fig. 17) developing into a compound foot (Figs. 20, 22). What is, however, relevant here is that during the trichogenesis, in the trichome initial which acts as the basal cell of the developing trichome, the nucleus makes the same to and fro movements (as described in *Clitoria*), the forward move giving rise to daughter cells that contribute to the formation of stalk and head, whereas the rear, unlike in *Clitoria*, being not a blind one is usually followed by anticlinal divisions (Fig. 17) giving the compound foot. However, since the foot cell of the 2-armed hair is of a shorter length as compared to that in *Clitoria*, the to and fro distances traversed by the nucleus in the initial are not conspicuous. It is relevant that in the head cell of the 2-armed hair also, the nucleus makes to and fro movements (Figs. 15-18). Nuclear movements recorded above are also common in the development of numerous other multicellular trichome types as described by Ramayya<sup>8</sup>.

From the observations described it could be stated that the larger the cell, the more conspicuous is the nuclear movement as seen in the foot cell of the filiform hooked hair in *Clitoria* and the head cell in the 2-armed hair of *Chrysanthemum*, but it is negligible in smaller ones as in the stalk cells of the latter trichome (Figs. 18-20). Indeed nuclear translocation may not be necessary in smaller cells since no region in such cells would remain beyond serviceable distance from the nucleus regarding supplies of its essential products.

The findings, particularly those from the ontogeny of the 2-armed hair in *Chrysanthemum* are important in shedding light on the biological value of the nuclear movements. Since the forward move of the nucleus in the basal cell is followed by the cell division, it is clearly indicative of its importance in bringing about cell divisions that lead to formation of the stalk and head of the trichome towards its distal end. Similarly, the rear move of the nucleus is followed by cell divisions giving rise to the compound foot. In instances where the nucleus

on return does not divide as in some trichomes of the 2-armed hair of *Chrysanthemum* (Figs. 19, 21), or regularly in the filiform hooked hairs of *Clitoria* (Fig. 8), this shows that in these trichomes, though the nucleus is induced to move back, the capacity for cell division is lost. Thus the nuclear movements recorded suggest to be for the purpose of cell divisions at a prescribed site unlike those occurring during fertilization or secondary nucleus formation in embryo sac of angiosperms where they result in internuclear fusion. In the development of root hairs also, as they elongate, the nucleus maintains a corresponding forward movement<sup>11</sup>, but this is said to help in maintaining their tip growth<sup>11</sup>. In this instance, therefore, the nuclear movement helps in growth rather than leading to cell division. In some instances, however, nuclear movement is not suggestive of any specific morphogenetic or functional purpose. For example, this has been recorded in the case of the vegetative nucleus of pollen tubes and also synergid nuclei of some plants wherein they have been observed to simply wander in the upper end of the embryo sac<sup>5</sup>.

The present data are also significant in throwing light on some aspects of the causation of the intracellular nuclear movement. It is well known that directed movement of sperms towards eggs occurring during plant fertilization are considered to be chemotropic in nature<sup>4</sup>. Since the nuclear movements leading to cell division during development of the 2-armed hair and filiform hooked hair is directional and regulated in space and time, the authors regard this to be equally chemotropic, presuming the movement inducing stimulus to originate from the prospective site of cell division. In this context the blind rear movement of the nucleus observed in the basal cell of the filiform hooked hair is significant in as much as it provides some insight into the nature of the relationship between the nuclear movement and cell division. Normally these two phenomena occur together in close succession, the nuclear movement being followed by cell division as observed during the ontogeny of the two trichome types. But since in the basal cell of the filiform hooked hair the rear nuclear movement is not followed by cell division, it indicates that the movement inducing stimulus is distinct and separate from those causing cell division. Thus the cell divisions in the trichomes studied are indicated to be governed by several chemical stimuli at various levels acting in close co-ordination,

In retrospect the major events pertaining to the occurrence of intracellular nuclear movements observed in the trichomes studied can be resolved into the following: (1) origin of nuclear movement inducing stimulus, (2) movement of the

nucleus to the site of cell division, (3) organization of the cytoplasmic site to undergo division and (4) occurrence of cell division.

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### INFLUENCE OF SCORPION VENOM ON ENZYME SYSTEMS OF SCORPION *HETEROMETRUS FULVIPES*

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#### ABSTRACT

Presence of cannibalistic behaviour amongst scorpions was observed. Addition of venom to the hepatopancreatic homogenate had no influence on the activity of succinate dehydrogenase while glutamate dehydrogenase was elevated. In the homogenate of cephalothoracic neuronal mass, the venom inhibited succinate dehydrogenase and increased glutamate dehydrogenase. These differential effects of venom are discussed.

#### INTRODUCTION

AN observation of cannibalistic behaviour in the scorpion, *Heterometrus fulvipes*, where one scorpion injects its venom and immobilizes the other, prompted us to study the effects of scorpion venom on the enzyme systems of scorpion tissues. Venom from different species of scorpions were shown to have six protein fractions<sup>3-6</sup>. Oommen and Kurup<sup>4</sup> have suggested that the toxicity exists in the fractions having cathodic mobility and the relative toxicity of the venom depends on the proportions of the cathodic protein fractions<sup>7</sup>. Earlier investigations in our laboratory have shown that the administration of scorpion venom into cockroach inhibits respiration and decreases body temperature, succinate and lactate dehydrogenase, and acetylcholinesterase activity levels in the muscle and ventral nerve cord<sup>8</sup>. However, no reports are available on the effects of scorpion venom on its own tissues. Two enzymes were chosen as

representatives of oxidative and amino acid metabolisms.

#### MATERIALS AND METHODS

Scorpions were collected from local hilly terrain and were adapted to the laboratory conditions. They were kept in separate glass jars and were fed daily with cockroaches.

Venom was collected from freshly collected animals by applying electric shocks upto 15 V in the post-abdominal region with an Electronic Stimulator (Seemax, ST-5, Ambala). The venom was collected into a syringe and diluted with pH 7.4 (0.05 M) phosphate buffer. Protein level (2 mg/ml) was used as a check to obtain same dilution every time. Fresh venom was collected for each experiment.

Hepatopancreas and cephalothoracic nerve mass (referred as brain) were isolated from scorpions. Tissue homogenates were prepared in ice-cold 0.25 M sucrose solution in Potter-Elvehjem glass homogenizer. The homogenates were centrifuged

\* To whom the reprint requests should be made.

at 3000 rpm for 15 min. and the supernatant was used for assay.

Succinate dehydrogenase (succinate: acceptor oxidoreductase E.C. 1.3.991) and glutamate dehydrogenase (glutamate: NAD oxidoreductase E.C. 1.4.1.3) were assayed by modified dye reduction method<sup>1</sup>. 2.0 ml of the reaction mixture contained 50  $\mu$ moles of sodium succinate or 100  $\mu$ moles of sodium-L-glutamate, 100  $\mu$ moles of pH 7.4 phosphate buffer, 4  $\mu$ moles of INT [2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride] and 0.1  $\mu$ mole of NAD (for glutamate dehydrogenase only). The reaction was started by adding 0.2 ml of 10% (W/V) hepatopancreatic homogenate or 0.5 ml of 1% brain homogenate. The incubation was carried out for 30 min. at 37° C, and the reaction was stopped with 5 ml of glacial acetic acid. The formazan formed was extracted overnight into 5 ml of toluene and the

intensity of the colour was measured at 495 m $\mu$  with Bausch and Lomb Spectronic-20.

The experimental tubes received 0.1 ml of the venom while control tubes received same amount of distilled water. In preincubation studies, the homogenate was preincubated with venom and 100  $\mu$ moles of phosphate buffer (pH 7.4) for 10 min., and the aliquots were assayed for enzymic activity. Protein was determined by the method of Lowry *et al.*<sup>2</sup>.

#### RESULTS AND DISCUSSION

Succinate dehydrogenase of hepatopancreas was little affected by the venom while the brain enzyme was inhibited (Table I). The per cent inhibition was increased by preincubating the homogenate with venom and substrate could not protect the enzyme (Table I). Glutamate dehydrogenase activity was elevated by venom in both the tissues, but the

TABLE I

*Levels of succinate dehydrogenase activity (expressed as micro moles of formazan/mg protein/hr) in normal and venom treated tissues of scorpion*

	Hepatopancreas		Brain	
	Mean $\pm$ S.D.	Percentage Change	Mean $\pm$ S.D.	Percentage Change
Control	0.440 $\pm$ 0.004	Nil	0.326 $\pm$ 0.004	Nil
Homogenate plus venom	0.445 $\dagger$ $\pm$ 0.007	Nil	0.157* $\pm$ 0.006	-50.9
Homogenate plus Succinate plus venom (preincubated)	0.440 $\dagger$ $\pm$ 0.006	Nil	0.114* $\pm$ 0.003	-64.3
Homogenate plus venom minus Succinate (preincubated)	0.435 $\dagger$ $\pm$ 0.005	Nil	0.094* $\pm$ 0.005	-70.6

S.D. = Standard Deviation, \* = Statistically significant  $P < 0.01$ .

$\dagger$  = Statistically Non-significant. Number of samples tested, five in each case.

TABLE II

*Levels of glutamate dehydrogenase activity (expressed as micro moles of formazan/mg protein/hr) in normal and venom treated tissues of scorpion*

	Hepatopancreas		Brain	
	Mean $\pm$ S.D.	Percentage Change	Mean $\pm$ S.D.	Percentage Change
Control	0.116 $\pm$ 0.015	—	0.042 $\pm$ 0.0004	—
Homogenate plus venom	0.205* $\pm$ 0.006	+ 76.7	0.073* $\pm$ 0.0007	+ 73.8
Homogenate plus venom minus glutamate (preincubated)	0.266* $\pm$ 0.005	+ 120.6	0.059* $\pm$ 0.0004	+ 40.4

S. D. = Standard Deviation, \* = Statistically significant  $P < 0.01$ .

Number of the samples tested were five in each case.

per cent elevation was different in the two tissues during preincubation (Table II).

The observed differential effects of the venom in the two tissues may be due to (a) the tissue specific differences in the enzyme systems and/or (b) the possible presence of a detoxifying mechanism in hepatopancreas which nullifies the toxicity of the venom thereby the oxidative enzymes are little affected and the absence of such a mechanism in the neuronal tissue eventually makes the venom, a neurotoxin. Lack of substrate protection in succinate dehydrogenase suggests the possibility that the venom is acting at a site other than the active site.

A drop in succinate dehydrogenase may lead to a drop in the overall oxygen consumption of the neuronal tissue thus resulting in the anoxic state which may ultimately inhibit the total neuronal activity. The cessation of action potentials was observed by earlier investigators in the ventral nerve cord of cockroach<sup>3</sup>. The elevation of glutamate dehydrogenase activity in both the tissues suggests the stimulation of ammonia production, which by itself is lethal<sup>8</sup>. Due to the depletion of energy sources by decreased oxygen consumption, ammonia may not be metabolized further<sup>8</sup>. Further, the increased activity of glutamate dehydrogenase and

decreased oxygen consumption may affect the ratio of NADH/NAD, which influences the energy charge of the cell<sup>9</sup>. Thus it appears the venom exerts differential effects on different tissues.

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## LETTERS TO THE EDITOR

### ON THE PROBLEM OF RESISTIVITY VARIATION IN ANTIMONY DOPED SILICON CRYSTALS

THE resistivity variation along the growth direction in the pulled crystal is basically due to the fact that most of the impurities present intentionally or unintentionally in the silicon charge have their distribution coefficient

$$K = \frac{\text{concentration in solid}}{\text{concentration in liquid}}$$

much less than unity.

To minimise this variation along the length, methods like floating crucible method<sup>1</sup> have been suggested. However, floating crucible method has not been applied very successfully for silicon crystals. To some extent this variation along the length can be reduced by adjusting the effective distribution coefficient ( $K_{eff}$ ) which depends upon various parameters such as rotation, etc., of the crystal growth conditions<sup>2</sup>.

One can anticipate qualitatively the variation along length if only one kind of known impurity is present in the silicon charge. However, if more impurities than one are present, then an unusual variation of resistivity may take place along the growth direction.

In silicon industry the most important doping impurities are boron for *p*-type conductivity and phosphorous or antimony for *n*-type conductivity. Among these impurities, the distribution coefficient of boron in silicon is nearly unity and so it poses no problem of resistivity variation along the length of the crystal. Similarly the distribution coefficient of phosphorous is also quite high ( $\approx 0.35$ ) and to some extent in this case also the problem of resistivity variation is not serious. However, in the case of antimony for which the distribution coefficient is very small ( $\approx 0.023$ ), the resistivity variation is too high. This reduces the yield of useful length of the grown ingot to a large extent. To the best of our knowledge, there is no method reported at least in the literature to overcome this problem.

The technique of reducing the resistivity variation in antimony doped silicon crystals is reported in this paper. The crystals are pulled from the melt in a standard commercial Czochralski equipment. All the crystals were pulled in  $\langle 111 \rangle$  direction. Rate of pulling was maintained within the range of 0.1 mm/min to 1.5 mm/min. The speeds of

rotation of the crucible and seed were 5 and 10 revolutions per minute. The crystals were grown in the atmosphere of pure argon and hydrogen mixture.

During experimental studies on doping of silicon crystals at SSPL, it had been observed that the problem of resistivity variation in the case of antimony doped silicon crystals can be solved to much satisfaction by adopting a small trick of adding a very small percentage of phosphorous together with the antimony dopant. For example, Curve No. I in Fig. 1 shows the resistivity variation in antimony doped silicon crystal without addition of phosphorous while Curve No. II shows the variation in resistivity when the antimony dopant contains 0.5% phosphorous. This is a very satisfactory solution from device point of view, because both antimony and phosphorous are *n*-type dopants and secondly such a small percentage of phosphorous is not going to affect the purpose of antimony doping from the device point of view.

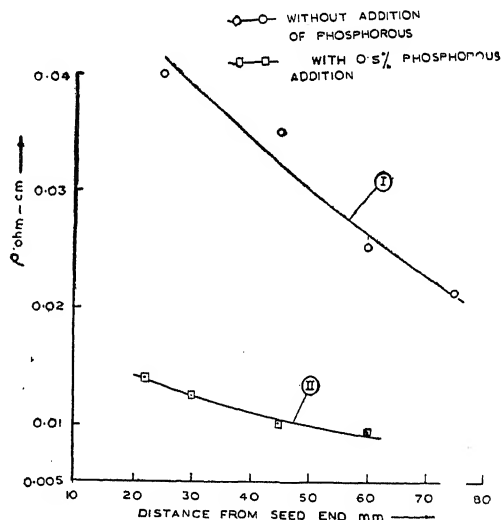


FIG. 1. Resistivity variation in antimony doped silicon crystals.

This experimental observation gives rise to one very important conclusion; that is, the distribution coefficient of antimony in silicon changes drastically in the presence of even very small quantities of phosphorous. To understand this thoroughly, further deep studies are essential in this field.

The curves in Fig. 1, are plotted for lower ranges of resistivities because these are the levels of anti-



mony doping generally used for the silicon crystal substrates used in integrated circuit devices.

The authors are indebted to Dr. S. C. Jain, Director, for his constant encouragement.

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### CRYSTAL STRUCTURE OF LYSINE ASPARTATE

It is well known that the structure and assembly of proteins critically depend upon non-bonded interactions. The atomic details of these interactions cannot, however, be understood from protein crystallographic studies on account of the limited resolution of protein electron density maps. Therefore, X-ray investigations of crystalline complexes among amino acids and their derivatives have been initiated. As the first stage in this programme, we have taken up the study of the complexes between basic and acidic amino acids. Here we report a preliminary account of the structure analysis of one of these, namely, L-lysine L-aspartate.

The compound, obtained commercially, was crystallized by slow evaporation of its aqueous solution at room temperature. The crystal belongs to the monoclinic space group  $P2_1$  with the following unit cell dimensions.  $a = 5.555 \pm 0.006$ ,  $b = 7.867 \pm 0.006$ ,  $c = 15.376 \pm 0.015$  Å and  $\beta = 99.1 \pm 0.1^\circ$ . The density ( $1.412 \pm 0.005$  g cm $^{-3}$ ) measured by flotation in a mixture of benzene and carbon tetrachloride agrees with that calculated ( $1.398$  g cm $^{-3}$ ) for two lysine aspartate units in the cell. The X-ray diffraction pattern was recorded on multiple-film equi-inclination Weissenberg photographs and the intensities were estimated by visual comparison with calibrated strips. The structure was solved by non-centrosymmetric direct methods<sup>1</sup> followed by conventional Fourier techniques. The atomic parameters were refined isotropically to a current R value of 0.165 for three-dimensional data. The positional coordinates and isotropic temperature factors of the atoms at the present stage of refinement are listed in Table I. Superimposed sections of the three-dimensional electron density map are shown in Fig. 1.

TABLE I

Positional and thermal parameters

Atom	x	y	z	B
N1	0.7179	0.2125	0.9015	1.9
C2	0.6091	0.3766	0.9370	2.3
C3	0.3416	0.3301	0.9514	2.1
O4	0.2347	0.2147	0.9029	2.7
O5	0.2611	0.4162	0.0070	2.5
C6	0.5892	0.5197	0.8683	2.1
C7	0.8438	0.5937	0.8561	2.4
C8	0.8208	0.7316	0.7856	2.4
C9	0.0579	0.8412	0.7948	2.4
N10	0.0760	0.9394	0.7107	2.2
N11	0.4436	0.3429	0.5067	1.7
C12	0.4271	0.3586	0.6036	1.7
C13	0.5427	0.1964	0.6497	1.7
O14	0.6901	0.1113	0.6107	2.1
O15	0.4865	0.1645	0.7241	2.7
C16	0.1665	0.3820	0.6174	2.0
C17	0.0703	0.5708	0.5885	2.4
O18	0.2208	0.6907	0.6629	2.8
O19	-0.1502	0.5786	0.5507	4.3

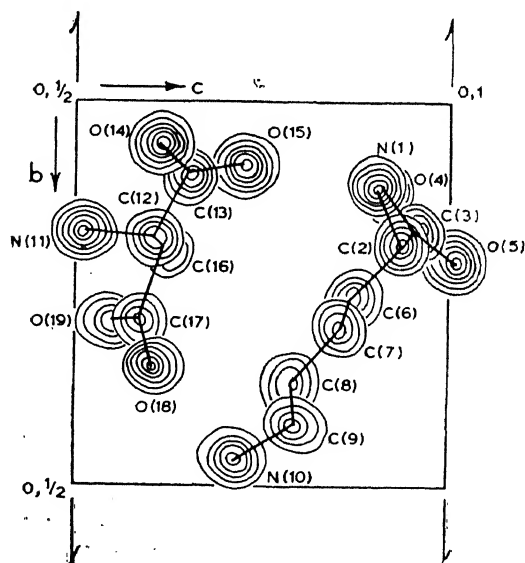


Fig. 1. Superimposed sections of the electron density map as viewed along the  $a$  axis. The contours start at  $2 e \text{ \AA}^{-3}$  and are at the same intervals.

The bond length and valency angles in the structure are normal. The conformation of the lysine and aspartate ions can be described by the following dihedral angles<sup>2</sup>.

Lysine :  $\psi_1 = 154^\circ$ ,  $\psi_2 = 332^\circ$ ,  $\chi^1 = 289^\circ$ ,  $\chi^2 = 178^\circ$ ,  $\chi^3 = 162^\circ$ ,  $\chi^4 = 161^\circ$ .

Aspartate :  $\psi_1 = 199^\circ$ ,  $\psi_2 = 21^\circ$ ,  $\chi^1 = 287^\circ$ ,  $\chi^{21} = 142^\circ$ ,  $\chi^{22} = 324^\circ$ .

The structure is stabilized primarily by ionic interactions involving amino and carboxylate groups.

Further refinement of the structure is in progress.

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### CHLOROCOALTATE (II) COMPLEXES FEATURING MORPHOLINIUM AND ETHYLENEDIMORPHOLINIUM CATIONS

CONSIDERABLE work<sup>1-4</sup> has been done on the anionic chlorocomplexes of divalent cobalt featuring group IA and bulky organic amine onium cations. In most of the cases studied, cobalt (II) has been assigned to possess a tetrahedral environment of chlorides. In the present study the cations employed, morpholinium (MH<sup>+</sup>) and ethylenedimorpholinium (EDMH<sub>2</sub><sup>++</sup>) ions, are both bulky and nonspherical differing between themselves in size and charge. It was considered interesting to observe the influence of these factors on the complexes.

*Dimorpholinium Tetrachlorocobaltate (II) (MH<sup>+</sup>)<sub>2</sub>* [ $\text{CoCl}_4$ ]<sup>2-</sup>.—3 gm of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 3.1 gm of morpholine hydrochloride were ground together when a semisolid resulted. This was agitated for an hour and kept at 100° C for two hours to remove most of the water and subsequently dissolved in hot methanol and the solution cooled in an ice and salt mixture. Ether was added to this to initiate precipitation of the complex and the blue crystalline solid thus obtained was filtered and washed several times with acetone-ether mixture and dried under vacuum at 30° C. Analysis: Expected for  $\text{CoCl}_2 \cdot 2\text{MHCl}$ , Co = 15.63% and Cl = 37.61%; Found: Co = 15.59% and Cl = 37.68%. The compound was slightly hygroscopic and on long exposure to humid atmosphere turned to pink solid.

*Ethylenedimorpholinium Tetrachlorodiquocobaltate (II) (EDMH<sub>2</sub><sup>++</sup>)* [ $\text{CoCl}_4 (\text{H}_2\text{O})_2$ ]<sup>2-</sup> and *Ethylene-*

*dimorpholinium Tetrachlorocobaltate (II) (EDMH<sub>2</sub><sup>++</sup>)* [ $\text{CoCl}_4$ ]<sup>2-</sup>.—3 gm of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  was dissolved in minimum amount of methanol and mixed while stirring with aqueous methanol solution of 3.5 gm of ethylenedimorpholinium dichloride, whereupon pale pink crystals of the complex separated out. This was filtered and washed with acetone-ether mixture and finally with ether and dried. On exposure to atmosphere for over six hours at room temperature the crystals slowly started turning to blue and this conversion was faster when the solid was kept in an air oven at 90° C. The freshly prepared sample gave the following analysis. Expected for  $\text{CoCl}_2 \cdot \text{EDMH}_2\text{Cl}_2$ : Co = 13.42% and Cl = 32.28%; Found: Co = 13.39% and Cl = 32.33%.

The presence of water in the pink crystals was confirmed during its decomposition. The weight change occurring in the solid phase corresponded to the loss of two molecules of water. Analysis of the blue complex established the composition as  $\text{CoCl}_2 \cdot \text{EDMH}_2\text{Cl}_2$ . Expected: Co = 14.62% and Cl = 35.18%; Found: Co = 14.58% and Cl = 35.07%. The blue solid kept in a desiccator containing water did not turn to pink form. However, recrystallisation of the blue solid from water gave once again the pink complex. This perhaps suggests that the two water molecules in the pink complex are coordinated to cobalt.

*Magnetic Susceptibility Measurement and Diffuse Reflectance Spectra.*—The magnetic susceptibility measurements were done by the Gouy method at room temperature. Diffuse reflectance spectra of the solid complexes were measured on SP 700 Unicam Cambridge spectrophotometer provided with a reflectance attachment. (We wish to thank Prof. G. W. A. Fowles, University of Reading, U.K., for providing the spectra).

*Discussion.*— $\text{CoCl}_2 \cdot 2\text{MHCl}$  gave a molar conductance of 117 ohm<sup>-1</sup> cm<sup>2</sup> in 10<sup>-3</sup> M acetone solution at room temperature which suggests that the complex behaves as a mono-bivalent electrolyte in solution. It has a magnetic moment of 4.75 B.M. at 29° C which indicates that cobalt(II) exhibits tetrahedral stereochemistry in this compound. The diffuse reflectance spectrum of the solid complex gives absorption peaks at 15.6, 14.6, 6.6 and 5.8 kK which are characteristic of cobalt(II) in  $T_d$  symmetry.

Of greater structural interest are the chlorocomplexes of cobalt (II) featuring ethylenedimorpholinium cation. The pale pink crystals of the aquo complex, (EDMH<sub>2</sub><sup>++</sup>) [ $\text{CoCl}_4 (\text{H}_2\text{O})_2$ ]<sup>2-</sup> gave a magnetic moment of 5.13 B.M. at room temperature. On the basis of this and its colour the complex is assigned an octahedral structure where both

the water molecules are coordinated to cobalt in addition to the four chlorides. As mentioned previously, the dihydrate is unstable and gradually transforms itself to the anhydrous ethylenedimorpholinium tetrachlorocobaltate (II),  $(\text{EDMH}_2^{++})[\text{CoCl}_4]^{2-}$ . For this reason the reflectance spectrum of the aquo complex could not be obtained.

The anhydrous chlorocomplex, unlike the dihydrate, is intensely blue and gives a magnetic moment of 4.71 B.M. The reflectance spectrum of this complex is very much similar to that of tetrahedral  $\text{CoCl}_2 \cdot 2\text{MHC}$  and exhibits absorption maxima at 15.6, 14.5, 6.7 and 5.8 kK. Crystalline octahedral complex having the unit  $[\text{CoCl}_4(\text{H}_2\text{O})_2]^{2-}$  and its transformation to  $[\text{CoCl}_4]^{2-}$  has not been reported so far in the studies on chlorocobaltate(II) complexes.

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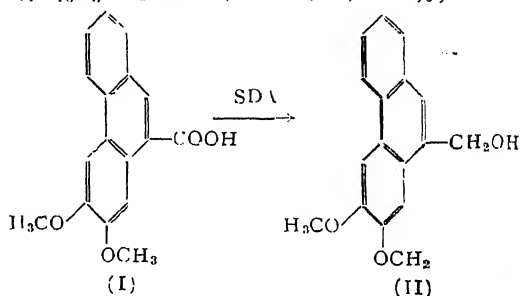
#### REDUCTION OF THE SUBSTITUTED PHENANTHRENE-9-CARBOXYLIC ACIDS BY SODIUM DIHYDRO-BIS(2-METHOXY-ETHOXY) ALUMINATE TO 9-PHENANTHRYLMETHANOLS

We required large quantities of 9-phenanthrylmethanol derivative for our work on the synthesis of phenanthroindolizidine derivatives as antileukemia agents<sup>1</sup>. Chauncy and Gellert<sup>2</sup> reduced the phenanthrene-9-carboxylic acid to 9-phenanthrylmethanol by using diborane. We report here the above reduction by using sodium dihydro-bis(2-methoxy-ethoxy) aluminate  $\text{NaAlH}_2(\text{OCH}_2\text{CH}_2\text{OCH}_3)_2$ , abbreviated as SDA, first synthesised by Vit, Casensky and Machacek<sup>3</sup>. The reduction is immediate in most of the cases reported and is invariably completed in an hour's time<sup>4</sup>.

#### EXPERIMENTAL

6, 7-Dimethoxy-9-hydroxymethylphenanthrene (II).—A solution of 6, 7-dimethoxyphenanthrene-9-carboxylic acid (I), (1.12 g 4 m mols) in hot dry tetrahydrofuran (10 ml) was added dropwise to a benzene solution of SDA (2.28 g, 8 m mols) in dry benzene (6 ml) with stirring. The reaction

mixture, which was protected from moisture, was refluxed with stirring for two hours. Water (50 ml) was added to the cold reaction mixture and the precipitated sodium aluminate was dissolved in dilute sodium hydroxide. The benzene layer was separated and the aqueous layer extracted with ether (40 ml). The combined benzene-ether extracts were dried over magnesium sulphate. The white residue left after removal of solvent was purified by chromatographing over neutral alumina using benzene as eluent and finally crystallised from benzene as colourless needles, m.p. 157°. Yield 0.66 g (63%). (Found: C, 76.17; H, 5.79.  $\text{C}_{17}\text{H}_{16}\text{O}_3$  required C, 76.12; H, 5.97%).



Similarly, phenanthrene-9-carboxylic acid; 6-methoxyphenanthrene-9-carboxylic acid; 2,3-dimethoxyphenanthrene-9-carboxylic acid; 2,3,6-trimethoxyphenanthrene-9-carboxylic acid and 2,3,6,7-tetramethoxyphenanthrene-9-carboxylic acid were reduced with SDA to the corresponding 9-hydroxymethylphenanthrene, m.p. 149°; 6-methoxy-9-hydroxymethylphenanthrene, m.p. 148°; 2,3-dimethoxy-9-hydroxymethylphenanthrene, m.p. 201°; 2,3,6-trimethoxy-9-hydroxymethylphenanthrene, m.p. 186° and 2,3,6,7-tetramethoxy-9-hydroxymethylphenanthrene, m.p. 184° in 60–75% yield. Mixed m.p. with the authentic samples of 9-hydroxymethylphenanthrene derivatives were not depressed.

Thanks are due to Professor S. Sethna for his interest in the work and Dr. S. S. Lele for microanalysis of the samples.

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# A SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF VANADIUM (IV)

IN connection with the solvent extraction of vanadium (IV) from acidic aqueous solutions, the need for a sensitive spectrophotometric method for

Red colour due to the formation of ferroin appeared instantaneously and after ten minutes the absorbance of the solution was determined in Bausch Lomb Spectronic 20 spectrophotometer at 510 nm. Values of absorbance at various concentrations of vanadium (IV) are listed in Table I.

TABLE I

Variation of absorbance of the reaction mixture with the concentration of vanadium (IV)

Concentration of vanadium (IV) $\times 10^5$ M ..	1	2	3	4	5	6	7
Absorbance ..	0.10	0.20	0.30	0.41	0.50	0.60	0.72

the estimation of small concentrations of vanadium (IV) was felt. Though there are some excellent methods for the spectrophotometric determination of vanadium (V)<sup>1-4</sup>, suitable methods for the estimation of vanadium (IV) are scarce in literature. Based on the recent kinetic study of the reversible reaction<sup>5</sup>

$\text{Fe (Phen)}_3^{3+} + \text{V (IV)} \rightleftharpoons \text{Fe (Phen)}_3^{2+} + \text{V (V)}$   
a method has been developed for the estimation of vanadium (IV) at ppm level. The second order rate constant was found to be  $5.7 \pm 0.1 \text{ M}^{-1} \text{ S}^{-1}$  at  $25^\circ \text{C}$  in 1 M sulphuric acid medium for the oxidation of vanadium (IV) by ferriin. Addition of acetic acid increased the rate appreciably ( $k = 25.1 \text{ M}^{-1} \text{ S}^{-1}$  in presence of 0.67 M sulphuric acid and 2.50 M acetic acid). By the proper choice of acetic acid concentration and addition of excess ferriin the reaction can be forced to go to completion as written. A mole of ferroin is thus produced for each mole of vanadium (IV) oxidized. Since the product ferroin has a high molar extinction coefficient at 510 nm ( $\epsilon = 11100$ )<sup>6</sup> where all the other species do not absorb, it is possible to determine accurately small amounts of the reactant vanadium (IV).

Ferric phenanthroline complex, i.e., ferriin (20 mM) was prepared from requisite amounts of ceric ammonium sulphate and ferroin. Ten ml of this solution was added to 20 ml of glacial acetic acid and made up to 100 ml to get a stock solution of ferriin (2 mM). Vanadium (IV) sulphate solution ( $\sim 15 \text{ mM}$ ) was prepared by dissolving a known amount of vanadium (IV) sulphate hydrate in distilled water. It was standardised by titrating with standard cerium (IV) solution<sup>7</sup>. Stock solution of vanadium (IV) (0.2 mM) was obtained by suitable dilution. Reagent grade chemicals (BDH) were employed throughout.

Different volumes of vanadium (IV) solution (0.5 to 3.5 ml) were added to 6.0 ml of ferriin solution and the volume made up to 10 ml with acetic acid and water in required amounts, so that the acetic acid concentration is 2 M in all solutions.

The plot of the optical density versus concentration of vanadium (IV) was a straight line passing through the origin. With the help of this calibration plot it was found possible to determine unknown concentrations of vanadium (IV) in the range  $1 \times 10^{-5}$ – $7 \times 10^{-5} \text{ M}$  (0.5 to 3.5 ppm) with good photometric accuracy (1 to 2%).

Interferences of various cations and anions was studied by adding the interfering radicals to the vanadium (IV) solution (2.5 ml) and determining the absorbance in each case. The variation in the absorbance was less than  $\pm 0.02$  units in presence of 1 mg of manganese (II), aluminium (III), cadmium (II), calcium (II), magnesium (II), lead (II), chromium (III), sodium (I), potassium (I) and uranium (VI). 50 mg of citrate, chloride, acetate and 25 mg of phosphate also did not interfere. Small amounts of copper (II), cobalt (II) and nickel (II) interfere. Oxidizing and reducing agents which interact with the reactants should be absent. Addition of excess of free ligand, *O. phenanthroline*, stabilizes the ferriin solution, and helps in practically eliminating the interference due to copper (II), nickel (II) and zinc (II).

Other systems involving the reaction between vanadium (IV) and ferric complexes of substituted *O. phenanthrolines* or bipyridyl would be useful in the determination of vanadium (IV).

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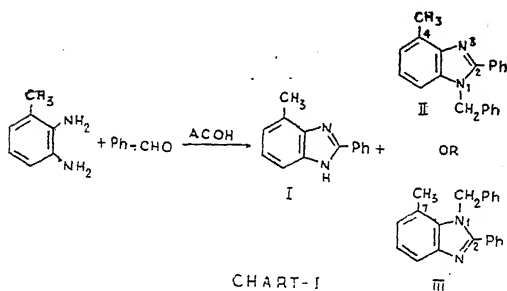
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CONDENSATION OF 3-METHYL-*o*-PHENYLENE  
DIAMINE WITH BENZALDEHYDE:  
FORMATION OF 4-METHYL BENZIMIDAZOLES

WHILE extensive work was carried out on the condensation of simple and 4-substituted *o*-phenylenediamines with aldehydes<sup>1</sup>, studies on a similar condensation with 3-substituted diamines has not so far been reported. In acetic acid medium, the diamine-aldehyde condensation is known to yield 2-mono and 1,2-disubstituted benzimidazoles.

In the present investigation, 3-methyl-*o*-phenylene diamine has been condensed with two moles of benzaldehyde in acetic acid resulting in two crystalline compounds (TLC pure), one melting at 250° and the other at 105°. The former has been characterised as 2-phenyl-4 (7)-methyl benzimidazole (I), which was earlier prepared by another method<sup>2</sup>. The latter on the basis of chemical analysis and ir and nmr spectral data has been considered to be 1,2-disubstituted benzimidazole-either 1-benzyl-2-phenyl-4-methyl benzimidazole (II) or 1-benzyl-2-phenyl-7-methyl benzimidazole (III). To ascertain the structure of the compound, (II) and (III) have been independently synthesised through unambiguous steps, starting from 3-chloro-2-nitro toluene and 2-chloro-3-nitro toluene respectively. The 1,2-disubstituted benzimidazole obtained in the aldehyde condensation could be identified as 1-benzyl-2-phenyl-4-methyl benzimidazole (II).

The formation of (II) but not (III) in this diamine aldehyde condensation through an expected dibenzylidene derivative will be favoured by electronic factors<sup>3</sup>. Full details of the work will be published elsewhere.



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EFFECT OF RICE TUNGRO VIRUS (RTV)  
INFECTION ON STARCH, REDUCING SUGAR  
AND PHOSPHORUS CONTENTS OF LEAVES  
OF DIFFERENT VARIETIES OF RICE

MOST of the high yielding varieties of rice are susceptible to rice tungro virus (RTV). Taichung (Native) 1 and Padma are highly susceptible whereas Jaya and IR-8 are moderately susceptible. A few workers 1, 2, 4 have observed alterations in the chlorophyll, starch and ribonucleic acid content in T(N) 1. Chowdhury<sup>3</sup> first observed different decrease of chlorophyll content and increase in RNA content in leaves of Padma, Jaya and IR-8 according to their relative susceptibility to RTV. In this report, attempt has been made to observe changes in starch, reducing sugar and phosphorus in RTV inoculated leaves of Padma, Jaya and IR-8.

The selected rice varieties were grown at the University farm in Kharif 1971, in replicative plots following usual cultivation practices. Nitrogen was applied at the rate of 40 kg N/ha in split dosage and no plant protection schedule was adopted. Field inoculation was done by releasing viruliferous *Nephotettix virescens* on T(N) 1 seedlings grown in two rows around each experimental plot. Uniform number of leafhoppers were released in each row so that the test plants may be exposed to the identical pressure of the inoculum. The plots were kept under continuous observation and leaf samples were collected from the diseased plants after 30 days of the appearance of symptoms. The samples were oven-dried at 80° C for 48 hours and then powdered with a grinder using 80 mm mesh. The starch, reducing sugar and phosphorus contents of different samples were estimated following the methods of Yoshida *et al.*<sup>5</sup>. To estimate reducing sugar and starch, the samples were treated with 80% ethanol. The supernatant obtained was used for sugar analysis while the residue left, was utilized for starch analysis. The residual material was treated with perchloric acid. The glucose content of the supernatant thus obtained and sugar solution previously collected were determined by anthrone reaction and subsequent spectrophotometric measurement of the absorbancy at 630 mμ. The glucose standards for sugar extract and starch extract were separately prepared following different procedures. Phosphorus content was determined by treating the acid extracted (mixture of nitric, sulphuric and perchloric acids) materials with nitric acid and molybdate vanadate solution and subsequent spectrophotometric measurement at 420 mμ, using monobasic potassium phosphate solution as standard.

Reducing sugar content decreased in the leaves of all the three varieties and was highest in Padma

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and lowest in IR-8. Starch content was found to increase in all the three varieties and the increase was highest in Padma and lowest in IR-8. In case of phosphorus content, a different pattern was observed. Phosphorus content was found to decrease in IR-8 and Jaya but to increase in Padma. The extent of decrease in IR-8 and Jaya markedly differed. It was very high in IR-8 and very low in Jaya.

From these observations it appears that reducing sugar content decreased and starch content increased due to RTV infection in Padma, Jaya and IR-8 and the extents of decrease and increase may be related to the relative susceptibility of these varieties.

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#### A MOSAIC DISEASE OF WATERMELON (*CITRULLUS VULGARIS* SCHRAD.)

A MOSAIC disease of watermelon (*Citrullus vulgaris* Schrad.) was found to be of common occurrence in the field plots of Horticulture Division of the Indian Agricultural Research Institute, New Delhi, in 1967. The disease has also been observed in the subsequent years. The chief symptoms of the disease are the diffuse mottling consisting of irregular light green areas on dark green background of the leaf (Fig. 1). Frequently young leaves show vein banding symptoms consisting of a narrow border of dark green along the veins and veinlets, the remainder of the leaf lamina being chlorotic. The older leaves show a more conspicuous mottling with dark green areas slightly raised in the form of blisters. The diseased vines set fewer and smaller fruits than healthy ones and are conspicuous because the tips of the runners protrude stiffly above the general level of the vines. The infected shoots show a shortening of internodes, resulting in

crowding of young leaves which appear somewhat stunted and rolled.



FIG. 1. Watermelon leaf showing Mosaic Symptoms.

The disease is readily mechanically sap transmissible to watermelon, cucumber, *Cucumis melo* (melon), *C. anguria*, bottlegourd, *Cucurbita pepo*, *Luffa acutangula*, tinda (*Citrullus vulgaris* var. *fistulosus*) and snakegourd (*Trichosanthes anguina*) inducing mosaic symptoms. Bittergourd (*Momordica charantia*) proved to be symptomless carrier and the virus caused chlorotic local lesions on *Chenopodium amaranticolor*. The virus could not be transmitted to other plant species outside family cucurbitaceae. None of the aphids, *Aphis gossypii*, *A. craccivora*, *Myzus persicae*, *Rhopalosiphum maidis*, *Brevicoryne brassicae* and *Sitobion rosae-formis* could transmit the virus and the virus is not seedborne in watermelon.

The virus has a thermal inactivation point of 95–98° C, a dilution end point between 1 : 10,00,000 and 1 : 50,00,000 and longevity *in vitro* of more than a year at room temperature. The virus was purified by the butanol centrifugation method as described by Nariani *et al.*<sup>6</sup> and ether carbon tetrachloride method described by Hollings and Nariani<sup>3</sup>. Both these methods yielded bluish grey, highly opalescent and infective virus preparations which in electron micrographs revealed rigid rod shaped virus particles with a modal length of 240 mμ (Fig. 2). The antiserum prepared by injecting rabbits intravenously and intramuscularly

with purified virus preparations was found to be specific and reacted with diseased plant sap in chloroplast agglutination and precipitin tube tests and had a titre of 1 : 2048. It reacted with bottle-gourd mosaic virus<sup>7</sup>. In reciprocal serological tests the watermelon mosaic virus reacted with bottle-gourd mosaic virus antiserum indicating their relationship.



FIG. 2. Electron micrograph of watermelon Mosaic virus particles,  $\times 34,000$ .

Bhargava and Joshi<sup>2</sup> isolated watermelon mosaic virus from *Cucurbita pepo* in Uttar Pradesh. The virus differs from the one reported herein in physical properties. Also it is aphid transmitted and is seed borne. The virus reported herein also differs from watermelon mosaic viruses reported by Anderson<sup>1</sup>, Van Regen Mortel *et al.*<sup>8</sup>, Lastra<sup>4</sup> and Milne and Grogan<sup>5</sup> which are long flexuous rods and aphid borne and have different physical properties. It, however, resembles in physical properties, host range and non-transmissibility through aphids and is almost similar in particle morphology to the bottle-gourd mosaic virus caused by *Cucumis* virus 2 reported by Shankar *et al.*<sup>7</sup>. In order to differentiate the watermelon mosaic virus from the one reported earlier from India<sup>2</sup> and the ones reported from abroad<sup>1,4-5,8</sup>, the name "vein banding mosaic" is proposed.

Grateful thanks are due to Dr. Nam Prakash of the Division of Agricultural Physics, I.A.R.I., for taking electron micrographs and to Dr. S. P. Raychaudhuri, Head of the Division of Mycology and Plant Pathology for his keen interest and encouragement throughout the course of these investigations.

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#### DISTRIBUTION OF SILICATE DISSOLVING BACTERIA IN VELLAR ESTUARY

It is well known that silicon dioxide forms an essential part of the skeleton in many silicoflagellates, diatoms, radiolarians and sponges. The availability of silicon in natural waters is facilitated by geochemical and biological cycling. The solubilization of the (normally insoluble) silicate minerals is one of the important steps affecting the silicon level in waters. It has been reported already<sup>1</sup> that increased solubilization of silicate minerals results wherever proteolysis takes place as they release basic amines. Silicate solubilizing bacteria isolated from the soil<sup>2,3</sup> and from the intestine of earth worms<sup>4</sup>, were found to be capable of dissolving insoluble silicates of calcium, magnesium and zinc<sup>3</sup>. Later reports<sup>5</sup> show that fungi are more efficient in the solubilization of silicates than either the bacteria or the actinomycetes. Though the role of silica in the productivity of natural waters are well known no attempt has yet been made to study either the distribution of silicate dissolving bacteria in marine environments or the role of such bacteria in the cycling of silicon in the sea water. The present study is an attempt to record the distribution of silicate dissolving bacteria in the sediments of Vellar estuary at Porto Novo, Tamil Nadu.

The present survey was carried out during the month of August, 1972. Sediment samples were collected with the aid of a Petersen grab from seven selected stations in the estuary covering the marine and fresh water zones. The salinity and

TABLE I  
Total and silicate dissolving bacteria in the sediments of Vellar estuary

Station No.	Description	Distance from Station 1 (km)	Type of sediment	Overlying water		Limnotolerant		Halotolerant	
				Salinity (‰)	Silicate ( $\mu\text{g/l}$ )	Total bacteria ( $10^5/\text{g}$ )	Silicate dissolving bacteria ( $10^5/\text{g}$ )	Total bacteria ( $10^5/\text{g}$ )	Silicate dissolving bacteria ( $10^5/\text{g}$ )
1.	Vellar mouth	0.0	Clay	35.0	475.8	4.53	2.20	5.92	1.86
2.	Killai mouth	0.5	Clay	34.0	363.1	2.77	0.92	2.98	0.92
3.	Biological Station	1.5	Clay	33.2	676.1	0.87	0.23	1.80	0.17
4.	Buckingham canal	3.0	Clay	32.2	951.5	8.65	2.74	6.54	1.27
5.	Railway bridge	4.5	Clay	29.4	1,458.6	9.28	3.33	9.52	2.85
6.	Mutlur	8.0	Sand	23.6	3,724.7	1.98	0.52	1.82	0.51
7.	Adanavur	10.5	Sand	14.0	8,807.8	1.90	0.48	1.17	0.22

silicate content of the overlying water were estimated following the methods of Strickland and Parsons<sup>6</sup> and Mullin and Riley<sup>7</sup>. The limnotolerant and halotolerant bacterial groups in the sediment samples were estimated using the appropriate media with different salinities<sup>8</sup>. For the enumeration of silicate dissolving bacteria, media supplemented with 0.25% magnesium trisilicate were used. Silicate solubilizing bacterial colonies were identified through the clear solubilizing zones around them (Fig. 1).

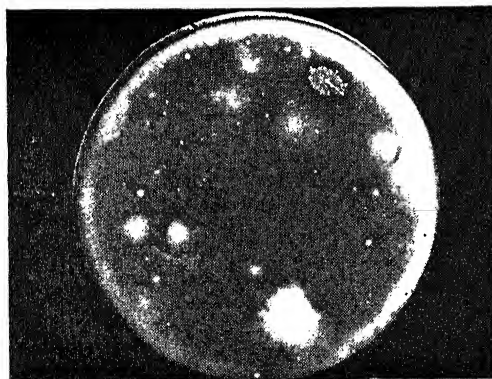


FIG. 1. Silicate dissolving bacterial colonies showing the characteristic solubilization zones surrounding them.

The results indicate that both limnotolerant and halotolerant silicate dissolving bacteria were present in all the samples irrespective of the variations in salinity (Table I). In general, limnotolerant silicate dissolving bacteria were found to be higher in numbers than the halotolerant group in all the

samples analysed. Clayey sediments always harboured more bacteria than the sandy ones. Majority of the isolates were found to be *Pseudomonas* spp. and the various isolates were also found to differ in the rate of solubilization of the insoluble silicates. It is now clear that the silicate dissolving bacteria have a definite distribution pattern in the marine environment and obviously they seem to have a definite role in the cycling of silicon in natural waters.

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# HOST PREFERENCE BY ADULT *EPILACHNA VIGINTIOCTOPUNCTATA* MOTSCH.

*Epilachna vigintioctopunctata* Motsch. popularly known as the lady bird beetle is one of the most serious pests of vegetable crops belonging to the *Solanaceae* and *Cucurbitaceae*.

From Table I it may be seen that potato was the most preferred. The order of preference among the vegetable crops was : potato > tomato > brinjal > cucumber > bean. Among wild hosts, the beetles showed the maximum preference for the phutka and the least for dhatura.

TABLE I

Showing host preference by the adult beetles of *E. vigintioctopunctata*, M.

No. released	Date	Number feeding on							
		Potato	Tomato	Brinjal	Cucumber	Bean	Wild host plants		
							Dhatura	Katrangni	Phutka
150	5-2-1973	36	9	22	..	7	4	12	38
150	6-2-1973	28	1	8	18	4	4	28	18
150	7-2-1973	28	30	4	2	4	1	21	37
150	8-2-1973	24	9	12	4	5	7	2	44
Total		116	39	36	24	20	16	63	137
Order of the host plants according to food preference		I	II	III	IV	V	III	II	I

Iwao (1953) carried out detailed studies on the food preference of *E. vigintioctopunctata*. According to him, the order of its food preference was as follows : (1) potato, (2) egg plant (brinjal), (3) tomato, (4) cucumber and (5) red pepper. Nakayama (1939) studied the host range of this beetle and reported that potato, egg plant, and phutka (*Solanum nigrum*) were its favourite food plants.

Due to the serious nature of this pest, investigations were made on host preference by adult *E. vigintioctopunctata*. The host plants included in the experiment were potato, tomato, brinjal, cucumber (*Cucumis sativum*), bean (*Dolichos lab lab*), dhatura (*Datura* spp.), phutka and katrangni (*Solanum xanthocarpum*). The twigs of different host plants were put in jars filled with water and the jars were placed inside the cage in a circular fashion. One hundred and fifty adults were confined in the cage each day and the number found feeding on each plant was counted and recorded after 24 hours. The experiment was repeated continuously for four days.

The results are presented in Table I.

The author is grateful to Dr. A. Ram, Principal and Regional Director, Agricultural Research Institute, Sabour, for providing necessary facilities. Agric. Research Institute, S. C. MANDAL. Rajendra Agricultural University, Bihar, Sabour, October 27, 1973.

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## ON THE METACERCARIA OF *PROSOGONOTREMA* (TREMATODA : PROSOGONOTREMATIDAE) FROM PLANKTON OF WALT AIR, BAY OF BENGAL

BOTH elasmobranchs and teleosts have been recorded as hosts for two species of *Prosogonotrema* by Ali and Bagwan<sup>1</sup> and Hafeezullah<sup>2</sup> respectively from the Arabian Sea (Bombay and Karwar). Yamaguti<sup>3</sup>, in his recent compendium on Digenea of the world reported that nothing is known about their life-history. No adults have yet been encoun-

tered in fishes of Bay of Bengal so far. This report indicates the possibility of a planktonic organism serving as a second intermediate host because of the recovery of the present specimen from a plankton collection from the inshore waters of Waltair during a summer. At the time of collection the parasite was still alive and active in sea water.

The larva (Fig. 1) is elongate measuring 2.08 mm in length and 0.55 mm in width. The oral sucker (OS) measures  $0.156 \times 0.187$  mm and the acetabulum (AC) situated at a distance of 1.26 mm from the anterior end is  $0.464 \times 0.414$  mm in diameter. The pharynx (PH) measuring  $0.097 \times 0.11$  mm leads into a short oesophagus which in

and *P. pritchardae* Hafeezullah, 1971 is described from *Gastrophysus spadiceus* (Richardson) (Tetradontidae) and *Pristopomoides argyrogrammicus* Val (Anthidae). Species from other regions of the world come from fishes as diverse as *Caranx* (Philippines), *Ocyurus* (Cuba, Jamaica), *Clupea* (Celebes), *Abalistes* (Gulf of Tonkin) and *Naso* (Hawaii). The report of *P. zygaenae* from the shark is interesting because all other reported hosts of the genus are teleosts.

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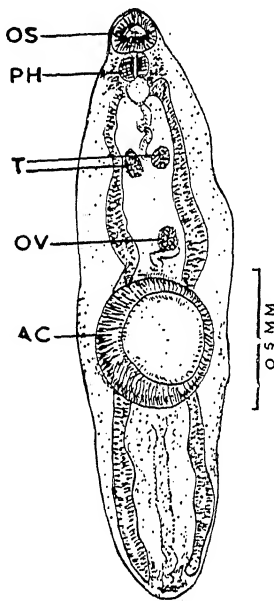


FIG. 1. Metacercaria of *Prosogonotrema*. PH: Pharynx, AC: Acetabulum, OS: Oral sucker, OV: Ovary, T. Testes.

turn opens into prominent sinuous caeca reaching the posterior end. Rudiments of the reproductive system consist of two juxtaposed testes situated behind the caecal bifurcation and measuring  $0.076 \times 0.048$  mm and  $0.06 \times 0.052$  mm respectively. A sinuous structure probably representing the vesicula seminalis lies anterior to the testes. A globular ovary (OV) 0.04 mm in diameter is situated antero-lateral to the acetabulum. These characters suggest that the larva belongs to the genus *Prosogonotrema*.

To date seven species of *Prosogonotrema* have been described. The genus seems to be of ubiquitous distribution both in geography and hosts. In India, *P. zygaenae* Ali and Bagwan 1971 comes from the hammerhead shark, *Zygaena malleus* from Bombay

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#### A NOTE ON THE SYNONYMY OF *GANEO* KAWI DWIVEDI AND CHAUHAN, 1970 WITH *GANEO BUFONIS* FOTEDAR, 1959

THE genus *Ganeo* was erected by Klein, 1905 for the forms which he collected from the intestine of *Rana hexadactyla* and designated it as *Ganeo glottoides*. A number of species have been added to this genus by various authors. In the present communication the author is concerned with two species, viz., *G. bufonis* Fotedar, 1959 and *G. kawi* Dwivedi and Chauhan, 1970; parasitizing the intestines of *Bufo viridis* and *R. tigrina* respectively. He considers *G. kawi* as a synonym of *G. bufonis*.

*G. bufonis* differs from *G. glottoides* in the absence of pseudocirrus sac and from *G. tigrinum* Mehra and Negi, 1928 in possessing an U-shaped excretory bladder without a median stem and complete absence of vitellaria on the right side of the body. Similarly *G. kawi* also differs from *G. glottoides* and *G. tigrinum* and also differs from *G. bufonis* in the complete absence of vitellaria on the left side of the body.

The position and arrangement of vitellaria in the genus *Ganeo* present many variations, specially in which the pseudocirrus sac is absent. If we consider *G. tigrinum* as type species for those in which the pseudocirrus sac is absent; the author noticed a number of variations in *G. tigrinum* itself like the grouping and non-grouping of vitellaria, converging of vitellaria towards the middle of the body, anterior or posterior extension or lessening of a number of ascini on any side or both sides. When all these variations are occurring within one species they become intraspecific variations and will not have any taxonomic importance.

Similar is the case with the absence of vitellaria on one side of any species and the author attributes this phenomenon to two reasons. One may be due to the non-development of vitellaria because of any abnormality in the normal developmental process and the other may be due to any mutations. Parasites during the process of life-cycle have to pass through a wide variety of environmental conditions and during this period they are prone to mutations. Such mutations will change the course of gene action, leading to a change in the morphogenesis and phenotype, and culminate in making the vitellaria absent on one side.

In view of the points discussed *G. bufonis* Fotedar, 1959 can become synonym of *G. tigrinum*, but the former retains its specific position in possessing the U-shaped excretory bladder without a median stem. *G. kawi* Dwivedi and Chauhan, 1970 differs from *G. tigrinum* on similar grounds but resembles *G. bufonis* in all respects except in the arrangement of vitellaria. Since the absence of vitellaria on one side is a variable character and its incidence is only 1 or 2%, the author gives no systematic importance to this character and considers *G. kawi* Dwivedi and Chauhan, 1970 as a synonym of *G. bufonis* Fotedar, 1959.

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#### COMPLETE INHIBITION OF VITELLOGENESIS AFTER EXTIRPATION OF MEDIAN NEUROSECRETORY CELLS IN *DYSDERCUS* *CINGULATUS*

EARLIER observations in our laboratory on *Dysdercus cingulatus* revealed that though there was some inhibition of vitellogenesis as a result of total extirpation of median neurosecretory cells (*mnc*), the animal laid some well-developed and apparently normal eggs<sup>1</sup>. It was suggested that this might be because the proximal region of the aorta was a neurohaemal organ and that the material stored in the aorta might have been released into the blood stream after extirpation of neurosecretory cells. The age of the insects used

for extirpation was between 6 to 30 hr after adult emergence which probably allowed the *mnc* to release the hormone into the blood. The present study was carried out to check this possibility and to confirm the role of the *mnc* by transplanting them into insects deprived of their *mnc*.

Adult females 0 to 3 hr after adult emergence were used for the present study. Surgical procedure for the extirpation of *mnc* and the histological techniques were as described already<sup>1</sup>. Ovaries were studied in a set of *mnc* extirpated insects and in sham operated animals which served as controls. The *mnc* of host insects were extirpated 0 to 3 hr after adult emergence and transplantation of *mnc* was performed two days after operation. For transplantation *mnc* of 3-day old normal females were used. These animals were anaesthetised with ether and *mnc* clusters with a little bit of surrounding tissue were dissected out from the brain and kept in insect Ringer. A slit was made on the abdominal tergite and the *mnc* from one animal were inserted through it into the abdominal cavity. 20 animals were used for this experiment. *mnc* extirpated hosts into which fat bodies were implanted served as controls. Some normal males were kept along with all the experimental and control females. The animals were fed on soaked cotton seeds. Survival rate was over 50%. Removal of *mnc* was confirmed by subsequent staining procedures and the data from those animals from which extirpation was incomplete were not considered.

Insects in which only the *mnc* extirpation was performed did not develop mature eggs even 16 days after the operation. Oocytes showed no sign of vitellogenesis. Resorption of oocytes occurred after 6 to 7 days. The sham operated controls developed and laid eggs within 7-8 days.

Out of 20 females implanted 8 died. The rest were active; ten showed swelling of the abdomen 2-5 days after transplantation indicating development of oocytes. On dissection ovary was found to contain 4-7 mature eggs in each ovariole. In the remaining two animals vitellogenesis did not take place. Mortality among controls was low. Ovaries of these *mnc* extirpated controls remained in the previtellogenic stage even after 6 to 7 days.

The present study reveals that vitellogenesis in insects from which *mnc* were extirpated 0 to 3 hr after adult emergence was completely inhibited. Hence the age of the insect at the time of extirpation of *mnc* appeared to be important as extirpation 6 to 30 hr after emergence only partially inhibited vitellogenesis<sup>1</sup>. This might be due to the fact that the release of neurosecretory material from *mnc* might have already taken place or considerable amount was already stored 6 to 30 hr

after emergence. Present studies confirm the importance of *mnc* in vitellogenesis as implantation of *mnc* from a reproductively and physiologically active female restores egg development in extirpated animals. Thus *Dysdercus cingulatus* is comparable to *Schistocerca gregaria*<sup>2,3</sup>, *Tenebrio molitor*<sup>4</sup> and mosquitoes<sup>5</sup> in *mnc* being essential for egg maturation.

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#### GERMINATION AND SEEDLING GROWTH OF RICE VARIETIES UNDER HIGH DEPTH OF SUBMERGENCE

Two newly recommended promising varieties of deep-water tracts of West Bengal, *Jaladhi 1* (selection from *Kalakherasail*) and *Jaladhi 2* (selection from *Baku*); two high-yielding varieties for low-lying areas, *Pankaj* and *Jagannath* and two well-known flood-resistant varieties, FR 13A and FR 43B, were studied to determine their varietal response with reference to germination and seedling growth under high depth of water. Seeds of these varieties were allowed to grow in tall cylindrical glass jars (32.0 cm × 8.0 cm) filled with water. The jars had 3 cm layer of clay loam soil at the bottom, over which seeds were spread, and covered with 1 cm layer of soil. Five seeds were sown per jar on 18th July 1973. Each variety was grown in two jars. The watering of the jars was commenced after sowing, with the addition of turbid water in the jars. The range of water temperature was 27–40° C. The experiment was carried out for 55 days. The results are presented in Table I and Fig. 1.

The seeds of all the test varieties germinated under submerged conditions in almost complete absence of oxygen. This nearly anaerobic condition existing during the seedling growth stage resulted in poor growth of the seedlings. The plants which survived showed certain structural changes such as the swelling of leaf sheath, the

formation of large aerenchymatous tissues, remarkable elongation of the culm and formation of narrow leaves.

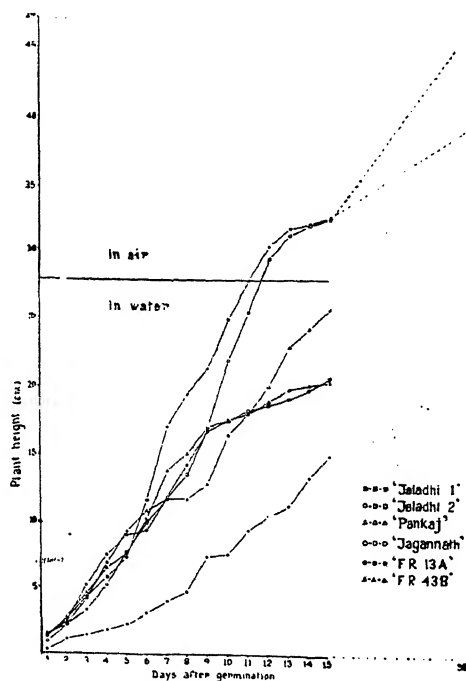


Fig. 1. Effect of high depth of submergence on the development of seedlings of the rice varieties.

Seedlings of deep-water varieties showed a higher degree of resistance to submersion and continued their rapid rate of growth in plant height until they emerged out from water but the flood-resistant and the high-yielding varieties did not come out above the water surface. The rates of growth of the deep-water varieties inside the water were very much higher (about 7 times) than in the air (Fig. 1). The results strongly suggest that the seedlings of deep-water varieties possess a greater degree of tolerance to anaerobic condition than the other types of varieties. Large air cavities were present in which air is stored for the respiration of rice plants<sup>1</sup>.

The maximum height of plants, was obtained in Jaladhi 2 (46.3 cm) and the minimum in FR 13A (21.5 cm). Jaladhi 1 and 2 showed no significant differences among themselves (Table I). The increasing trend of growth in height of the plant was steady, uniform and rapid upto the 6th day from germination (Fig. 1) in all the varieties except FR 13A. Thereafter a sudden rise in the rate of increase was noted in Jaladhi 1 and 2 which continued up to the 55th day from germination. Daily growth of as much as 5 to 6 cm was not

TABLE I

*Germination and seedling growth of rice varieties under submerged condition*

Variety	Germination period (day)	Days required to emerge out from water	Final height (cm)	Rate of growth/ day (cm)	
				in water	in air
<i>Deep-water varieties</i>					
'Jaladhi 1'	7	11	39.2	2.5	0.26
'Jaladhi 2'	7	12	46.3	2.3	0.40
<i>High-yielding varieties</i>					
'Pankaj'	7	..	26.2	1.7	..
'Jagannath'	7	..	23.3	1.5	..
<i>Flood-resistant varieties</i>					
'FR 13A'	7	..	21.5	1.0	..
'FR 43B'	7	..	23.1	1.2	..

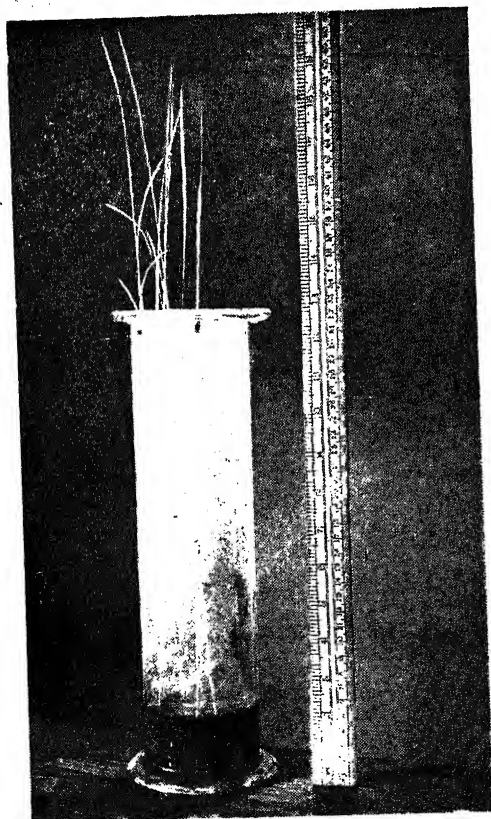


FIG. 2. Seedling growth of Jaladhi 2: young seedlings germinated and developed in water (photographed 55 days after germination).

infrequent when seedlings were growing in water. A relatively quicker rate of growth in height was noted in Jaladhi 1 inside water than in Jaladhi 2 till the 15th day after germination. But after their emergence from water, a rise in the rate of increase was noted in Jaladhi 1 which continued up to the 55th day from germination (Fig. 2). However, FR 13A and 43B exhibited an extraordinary capacity for submergence, i.e., 20–22 days. The high-yielding varieties, Pankaj and Jagannath, were found to be relatively less tolerant to complete submergence among the test varieties. The overall results indicate that there is a distinct biological as well as genetical difference among deep-water, flood-resistant and high-yielding varieties. The simple technique described can be profitably used in selection of promising varieties for low lying submerged rice areas.

The authors express their grateful thanks to A. K. Dutt, Director of Agriculture, West Bengal, and Shri D. K. Mukherji, Economic Botanist, West Bengal, for their keen interest in this work.

Salt and Flood Resistant

Paddy Scheme,

Rice Research Station,

Chinsurah, West Bengal, November 24, 1971.

S. K. DUTTA

B. BANERJEE

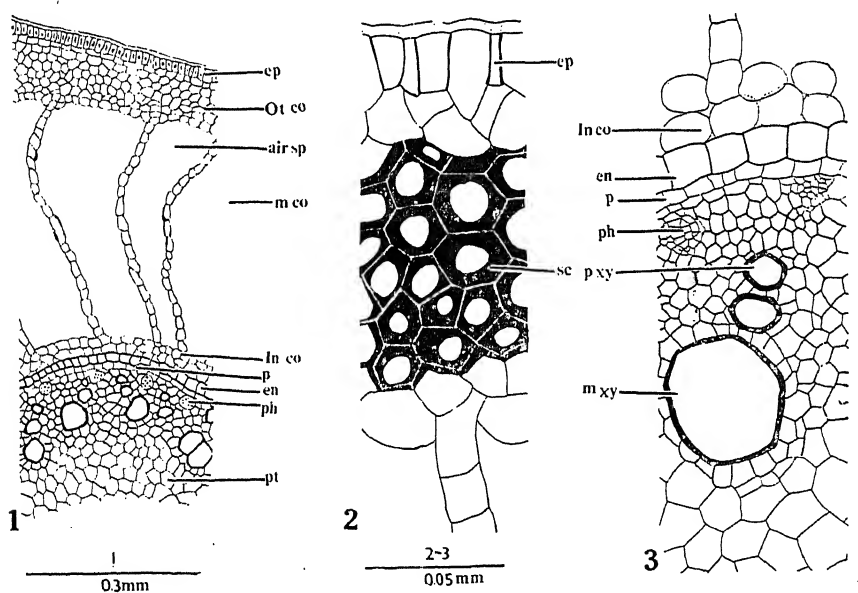
# OCCURRENCE OF FOSSIL *NYPA* ROOT FROM THE DECCAN INTERTRAPPEAN BEDS OF M.P., INDIA

GENUS *Nypa* in the fossil state has been reported by different workers from the Indian Tertiary strata<sup>1-3,5-8</sup>. So far, the genus has been known by its petiole and fruits only. This material of the *Nypa* root was collected by the author from the Deccan Intertrappean beds of Mohgaon kalan, M.P., India. On sectioning, a silicified block of chert revealed numerous roots which appear to resemble the extant *Nypa* root most closely.

The roots occur closely packed, they appear to be young and measure 2-3 mm in diameter. They are more or less circular in outline as seen in cross sections. The outermost layer epiblemma consists of very narrow cells which are somewhat elongated (Text-Figs. 1-2).

each other by very narrow partitions which are generally only one cell wide (Text-Fig. 1). Fibrous bundles are absent from this zone. Inner cortex is constituted by compact rows of thin walled cells, 2 to 4 layers thick (Text-Figs. 1, 3). Endodermis is only one cell thick, its cells are somewhat radially elongated, casparin strips cannot be made out (Text-Fig. 3). Immediately below the endodermis is present a single celled pericycle which is thin walled (Text-Figs. 1, 3).

The stele consists of 12-15 alternating xylem and phloem strands, all arranged in circle. Each xylem strand consists of one large vessel and one or two small vessels (Text-Figs. 1, 3). The smaller vessels are situated towards the periphery while the larger lie towards the centre (Text-Fig. 3). The large metaxylem vessels are  $66 \times 56 \mu$  in diameter, while the smaller protoxylem vessels measure



TEXT-FIGS. 1-3. Text-Fig. 1. A part of root in cross-section showing cortex and stele. Text-Fig. 2. A part of outer cortex with epidermal cells, showing sclerenchymatous tissue. Text-Fig. 3. A part of stele of the root showing xylem and phloem elements with endodermal and pericycle cells. (air sp, air space; en, endodermis; ep, epidermis; In.co.: inner cortex; m.xy, metaxylem; m.co, middle cortex; Ot.co, outer cortex; p, pericycle; ph, phloem; pt, pith; p.xy, protoxylem; Sc, sclerenchymatous cells.)

The cortex can be distinguished into three distinct zones, outer, middle and inner (Pl. I, Fig. 1). The outer cortex is about 5 to 6 cells deep, it is composed of closely packed row of dark coloured, thick walled cells (Text-Fig. 2). Thick walled outer cortex probably gave mechanical support to the root. The middle cortex is lacunose, it has air canals which extend radially from outer cortex to inner cortex. These air canals are separated from

$30 \times 33 \mu$  in diameter. Pith is made up of thin walled parenchymatous cells and have a central cavity (Pl. I, Fig. 2). The cells of this region are polygonal in shape. The medullary bundles are absent.

The root described above shows a very close resemblance with the root of *Nypa fruticans*, as described by Tomlinson<sup>10</sup>, in its epidermal cells. In addition, the cortex of this root is in three

distinct zones, with the middle cortex being lacunose, endodermis has no casparin strips and the phloem elements are small.

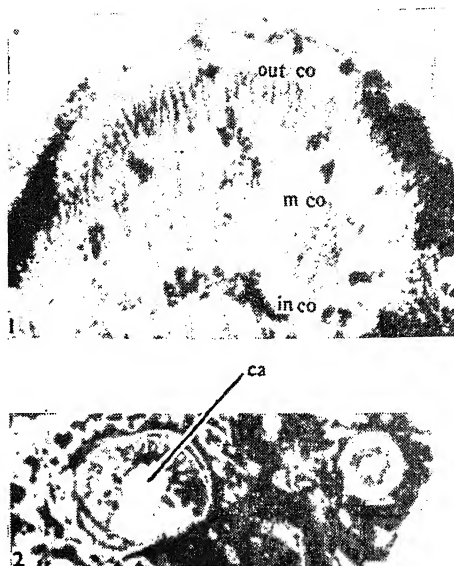


PLATE I

FIGS. 1-2. Fig. 1. A part of root in cross-section showing all the three zones of Cortex (*in co*; inner cortex, *m co*; middle cortex; *out co*, outer cortex),  $\times 25$ . Fig. 2. Stele of the root showing arrangement of xylem vessels and a central cavity (*ca*; cavity),  $\times 22$ .

*Nypa* root differs from other palm roots<sup>4</sup>, in having unthickened endodermis, small strands of phloem and cavity in the middle of the pith.

The fossil root described above shows some resemblance with the root of *Cyclanthodendron sahnii* but it differs in cortical structure and also in the structure of pith<sup>9</sup>.

As far as the author is aware the root of *Nypa* in fossil state is reported for the first time, though the fruits are of common occurrence in the intertrappean beds of M.P., India.

I am indebted to Dr. B. S. Trivedi, Reader in Botany, Lucknow University, for valuable suggestions and critically going through the manuscript. I am thankful to the Director, Birbal Sahni Institute of Palaeobotany, Lucknow, for Library facilities.

Department of Botany, C. L. VERMA.  
Lucknow University,  
Lucknow-7, January 13, 1973.

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#### A CASE OF REVERSION OF FLOWER OF *NYPHAEA MEXICANA* TO VEGETATIVE CONDITION

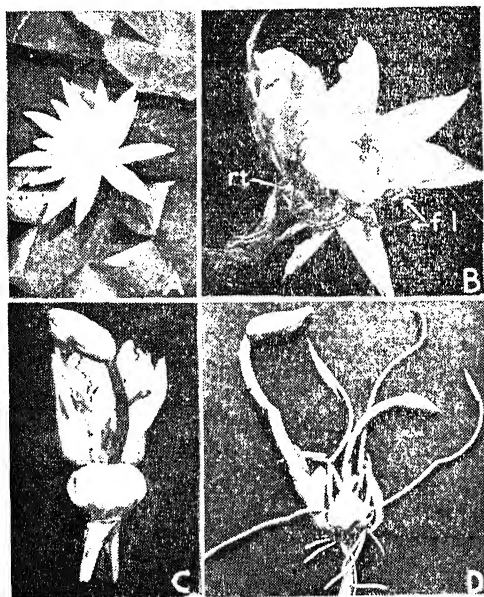
In a few plants of the yellow water-lily—*Nymphaea mexicana*, Zucc.—growing in the pond of the botanical garden of the University of Delhi, a certain number of flowers were found to show reversion to vegetative development.

The normal flowers, borne 3-4 cm above the level of water, are lemon-yellow in colour and open usually before noon during March to May. They have 5 free sepals, 23-25 spirally arranged petals which gradually become reduced in size and transformed into stamens. The latter are numerous and spirally arranged; the outermost are petaloid and bear anthers terminally; the innermost have narrow, short filaments. In certain stamens the anthers are placed unequally and in still others only one anther lobe was noted. The gynoecium is superior, multicarpellary (10-12 carpels), syncarpous, multi-locular, with numerous ovules in each locule (Fig. 1 A).

Some abnormal flowers were found floating on the surface of water. In these, the sepals and 5-8 outermost petals were similar to those in normal flowers, but the centre was occupied by a massive tuber-like structure. The latter was pinkish-yellow in colour, bearing foliage and scale leaves, nodulated outgrowths and clusters of roots. The foliage leaves were arranged in an acropetal succession and

the oldest had slender petioles measuring 25–30 cm in length. Each lamina was typical of the normal plants in shape but its two halves were infolded. Curiously, 2 or 3 flower buds were seen to arise in the position of petals, which were, however, normal and short-pedicelled (Fig. 1 D).

Foliage leaves, scale leaves and clusters of roots were observed to arise in the axils of petals in a few flowers (Fig. 1 B).



FIGS. 1. A–D. Fig. A. Part of the plant of *Nymphaea mexicana* showing a normal flower. Fig. B. An abnormal flower showing tufts of foliage leaves (*fl*) and roots (*rl*) in the axils of petals. Fig. C. 'Flower' showing accessory floral buds and multiple sex organs. Fig. D. An instance in which the reproductive parts are replaced by supernumerary floral buds, tufts of foliage leaves and roots.

In one flower three well-developed ovaries were present and each was surrounded by spirally arranged stamens, numerous petals but 5 sepals. A flower bud was seen to arise in the place of a petal (Fig. 1 C). Occasionally vegetative buds were initiated in the position of a flower.

While still attached to the mother plant, some of these metamorphosed "flowers" showed degeneration of the floral parts. Their foliage leaves and roots, however, grew vigorously. The latter were of two kinds: one firmly fixed in the mud at the bottom of the pond and the other free-floating. After a period of nearly one month these morphologically altered structures started descending due to increased weight. The roots became firmly fixed in the mud. The petioles of the foliage leaves elongated and lifted the laminae above water and

thus new plants were established. The extended observations of what appeared in the initial stages as flower anomaly led to the finding of a means of vegetative propagation in this plant.

What factors are responsible for the altered morphology of the flower is not clear. No pathogenic organisms were found to be associated with these structures. Hormonal imbalance may be another cause since under excised conditions flower buds are known to revert to vegetative state (Mohan Ram and Wadhi, 1966). Since in the same plants normal flowers were encountered but no fruit formation occurred, it could be tentatively hypothesized that the plants had adapted themselves to an alternative course of propagation for survival.

We are thankful to Dr. K. Subramanyam for his valuable comments. The award of a Junior Research Fellowship by the Bureau of Police Research, Ministry of Home Affairs, Government of India, to one of us (V. L. N.) is gratefully acknowledged.

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January 9, 1974.

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#### ASYNAPSIS IN *CHLOROPHYTUM LAXUM*

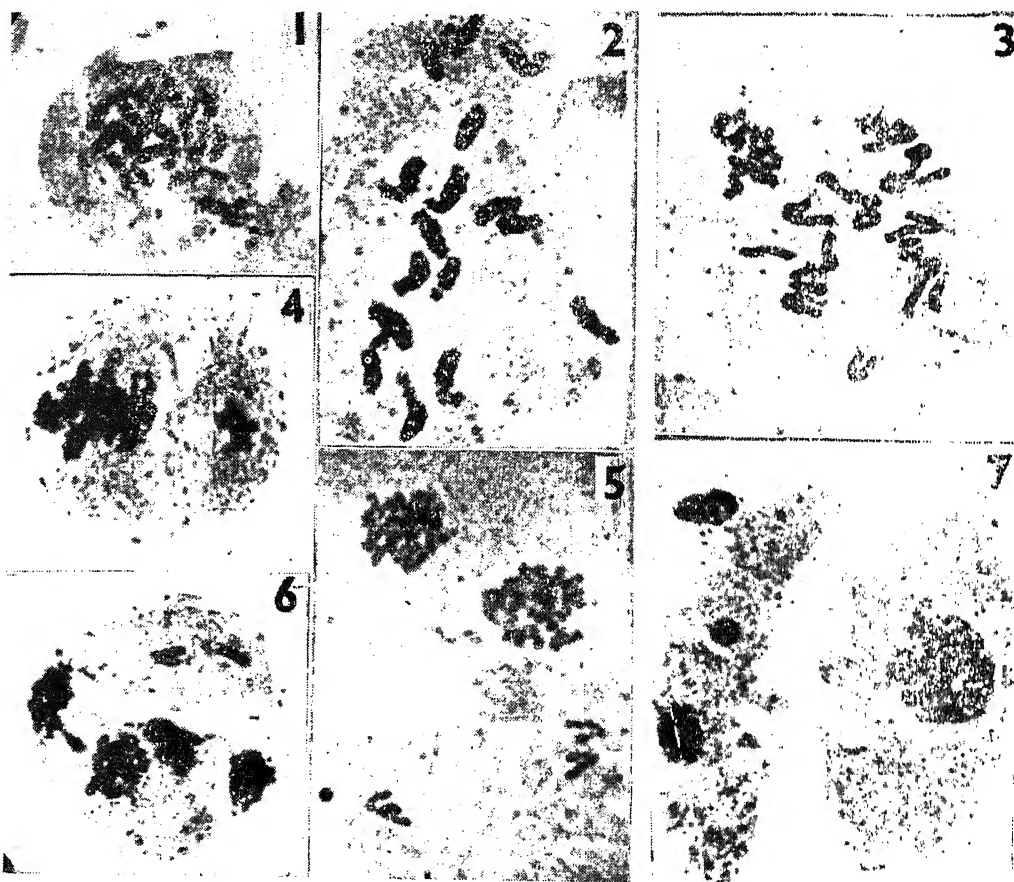
THE phenomenon of asynapsis and desynapsis of chromosomes during meiosis has been recorded in different organisms. Several causes, both intrinsic and extrinsic, have been attributed to it. In asynapsis, in contrast to desynapsis, there is complete failure of chromosome pairing and as such the univalent chromosomes would look like the somatic chromosomes. Li *et al.* (1945) suggested the restriction of the term desynapsis to cases where some amount of prophase pairing takes place and later the homologues separate out so that at diakinesis most of them are found as univalents. Some investigators are also of the opinion that the majority of cases under asynapsis have been found to be desynaptic, very few actually being asynaptic in the strict sense. Praaken (1943) has distinguished three types of desynapsis: weak, medium strong and complete desynapsis. Weak desynapsis is characterised by the occurrence of a few univalents in some cells as in *Mathiola incana* (Armstrong and Huskins, 1934), *Secale cereale* (Lamm, 1936), *Alopecurus pratensis* (Johnson, 1941), *Nicotiana sylvestris* (Goodspeed and Avery,



1939). Medium strong desynapsis is characterised by the formation of many univalents in most cells as reported in *Crepis capillaris* (Richardson, 1935), *Secale cereale* (Praaken, 1943), *Pisum sativum* (Kolier, 1938), *Nicotiana tabacum* (Clausen, 1931), *Zea mays* (Beadle, 1933) and *Solanum wendlandii* (Chennaveraiah and Krishnappa, 1968). In complete desynapsis, most of the cells reveal only univalents with some rare bivalents. This is seen in *Datura stramonium* (Bergner *et al.*, 1934), *Hevea brasiliensis* (Ramaer, 1934), *Alopecurus* (Johnson, 1944) and *Tradescantia* (Celarier, 1955), etc.

carmine squashes of pollen mother cells were made and the preparations were made permanent following the usual acetic acid-butyl alcohol series.

The course of meiosis is normal but in an isolated plant a variety of abnormalities arising from failure of synapsis was observed. Such a failure in all the six anthers had resulted in the formation of sixteen univalents (Figs. 1 and 2). They resemble somatic chromosomes although they appear somewhat condensed. As the first meiotic division proceeds it results in erratic behaviour of the univalents leading to various kinds of irregularities.



FIGS. 1-7. Figs. 1-2. PMC's with 16 univalents. Fig. 3. Division of the 16 univalents. Fig. 4. PMC at metaphase II with unequal number of chromosomes. Fig. 5. PMC at anaphase II. Figs. 6-7. PMC at telophase II, with micronuclei.

During the course of cytological studies in the Indian species of *Chlorophytum* asynaptic behaviour of chromosomes resulting in very interesting meiotic abnormalities was observed in *Chlorophytum laxum*. An account of these observations is presented in this paper. The material when flowering was collected from the open gardens of Bangalore (S. India) in the summer days of April. Aceto-

In some cases the sixteen univalents are found to even divide like mitotic chromosomes (Fig. 3) and move towards the opposite poles. The univalent chromosomes distribute randomly at anaphase I. Unequal distribution of chromosomes may result in a larger number of chromosomes in one of the daughter nuclei than in the other and consequently the former nucleus would be larger. This becomes

quite evident at the commencement of the second meiotic division (Fig. 4). During anaphase II the separation in such cells would result in equal distribution of the chromosomes, the larger nucleus forming two larger daughter nuclei and the two chromosomes of the smaller nucleus forming two smaller nuclei (Fig. 5). Such irregularities would result in the production of a variety of tetrad formations with unequal sized nuclei, micronuclei and polysporous condition (Figs. 6 and 7).

One of the fundamental questions about asynapsis or desynapsis relates to the cause or causes of these phenomena. Asynapsis has been attributed to several causes such as temperature, age, failure of chromosome spiralisation, etc. Praaken (1943) suggested that it may be due to gene action, loss of chromosome pairing, apomixis, and structural or numerical changes in chromosomes. Celarier (1955) and Ehrenberg (1949) are of the view that chiasmata formation may be the major factor involved in desynapsis. Celarier is also of the opinion that desynapsis may be due to inherited or environmental factors, the latter in turn influencing chiasmata formation. Chennaveeraiah and Krishnappa (1968) have suggested that desynapsis may be due to environmental and physiological conditions. Huskins and Smith (1934) reporting the occurrence of asynapsis in *Sorghum*, consider this phenomenon as due to premature splitting and irregular contraction of chromosomes. Genes causing complete asynapsis resulting in the production of polyploid gametes and offspring have been reported in corn (Beadle, 1930, 1933), wheat (Li *et al.*, 1945) and *Drosophila* (Gowen, 1933). Löve (1943) has described a case of  $\gamma$ -linked inheritance of asynapsis in *Rumex acetosa*. Krishnaswamy and Meenakshi (1957) have cited two types of desynapsis in the grain sorghums, genes of which behave as Mendelian recessives to the normal condition. Sinha (1967) has shown that asynapsis can be chemically induced in maize.

In the material under investigation, *C. laxum*, the observation that the asynaptic behaviour occurs as a rare phenomenon in stray plants in hot summer days is suggestive that it may be due to the effect of temperature acting as an environmental factor. However, the occurrence of such a phenomenon, although rare, assumes significance in connection with the rare occurrence of polyploids (tetraploids with  $2n = 32$ ) in *C. laxum* as reported by Sheriff and Nagaraj (1971). If asynapsis were to result in the production of viable diploid gametes it would provide a mechanism and account for the occasional existence of tetraploids in the taxon.

The authors wish to record their thanks to Prof. M. Nagaraj for the interest and facilities.

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#### OIL TANKER DISASTER IN NORTH-WEST COAST OF INDIA

OIL pollution in Indian Coasts has been a threat of increasing severity for the last few years<sup>1</sup>. On 18th June, 1973 threat became reality for the Gujarat Coast near Porbandar where the oil Tanker M.T. "COSMOS PIONEER" broke into two pieces releasing 18,000 tons of L.D.O. (Black oil). The tanker was on its way to Kandla from Bombay. The GRT of the tanker was 8996.75 tons and OAL was 505'10". During mishap of the tanker the Arabian Sea of Porbandar Coast was very stormy due to prevailing South-West monsoon and the wind velocity reaching upto 70-80 KM/hour. Experience with Torrey Canyon<sup>2</sup> disaster in whitesand Bay of Cornish Coast indicated of oil could be

predicted after the mishap only assuming that the oil moved relative to the water with a velocity vector about 3.3 to 3.4% of the relative wind vector. Oil spread from the illfated "COSMOS PIONEER" in an irregular pattern of streaks and patches changing with time and shaped by wind, tides and currents. Figure 1 indicates the movement of oil by surface currents soon after mishap.

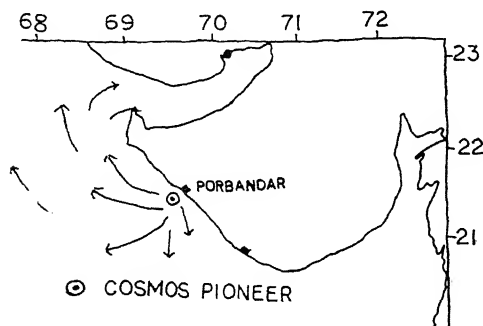


FIG. 1

Marine plants that were damaged were generally in high intertidal region, where the oil could remain on thalli, and dry during excessive tidal cycles. In more thickly oiled regions, the oil became less viscous when warmed by the sun (since it was mid summer) and ran down over new areas, after covering previously unpolluted plants. Damage to the barnacles of both rock and goose neck was extensive. Approximately 70% total population was killed. Barnacle mortality extended to the mid intertidal as a result of oil clinging to the rough surface of barnacles populations. All the dead individuals had a very heavy coating of oil over them and in many instances their cirri engaged in dried oil. Complaints were also received from local people that the fish they have purchased were inedible due to the possession of strong diesel fuel taint. In an attempt to see whether it could be positively identify the nature of taint, a sample of fish obtained from polluted water was sent to me. On being cooked the flesh was also tainted.

Of the total rock surfaces in some places, approximately 50% of the tops of rocks were covered with

tar along with the 25% to 30% of the sides. In certain areas the layer of tar was more than 4 cm deep. The oil in some places was heavy enough to form pools. The average concentration measured was 2.8 kg/m<sup>2</sup> at the high intertidal portions. The oil depositions in Gujarat Coast in space and time suggests that its effects on the marine biota will not be uniform. Areas differed greatly in the amount of pollution received, the oil in the lower and middle intertidal regions were intermittently covered with oil that in most cases were washed away within relatively short time. Damage to flowers and seeds may produce long term changes. In addition, the extensive plant and animal community associated with intertidal surf grass was undoubtedly affected in polluted areas and may take a considerable time to recover. There is however a risk of clean fish becoming tainted if the trawl becomes fouled during shooting or hauling.

In conclusion it should be emphasised that this preliminary study records only some of the more obvious and immediate effects of oil pollution. There is a clear indication that a subtle and gradual erosion of this natural source has begun. The gradual destruction of major coastal marine communities is probably being duplicated elsewhere along the north-west coast of India at the present time. It seems probable that repeated and continuing oil pollution will contribute to long term environmental degradation in the Gujarat Coast in addition to producing the short term effects noted in this present investigation. The detailed papers on the quantitative effects of oil on marine flora and fauna are now in progress and will be published in *Indian Fish Bulletin* in due course.

Offshore Fishing Station, V. D. RAMAMURTHY,  
Government of India,  
Old Kandla P.O. (Kutch).  
Gujarat, January 30, 1974.

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## SHORT SCIENTIFIC NOTES

### Detection of Loose Smut Infected Plants Before Ear Emergence in Wheat Cultivar Sonalika (RR-21)

Loose smut of wheat caused by *Ustilago tritici* (Pers.) Rostr. is an important problem in certified wheat seed production. Early removal and destruction of the infected plants before the smut spores are set free, are recommended. The disease is recognized after ear emergence when black powdery heads of infected plants appear. By the time such plants are rogued out, several spores might escape to adjoining plants before or during roguing and cause floral infections. This problem may be avoided if the infected plants could be detected before ear emergence.

It has been observed during the last three Rabi seasons (1971-72, 1972-73 and 1973-74) at Pantnagar that loose smut infected plants of wheat cultivar Sonalika (RR-21) could be recognized before ear emergence. The flag leaf of an infected plant before heading showed characteristic yellowing and formation of chlorotic streaks which later became necrotic. Such flag leaves started drying from the tip and took a brownish colour. Flag leaves of infected plants could easily be seen in the field from a distance. This relationship of a discoloured flag leaf and smutted head was repeatedly confirmed by examining numerous plants before and after ear emergence. All those plants with smutted heads, examined before or after ear emergence, showed characteristic flag leaves described above. It is suggested that early detection of infected plants prior to ear emergence could profitably be utilized in roguing diseased plants before spores are set free after ear emergence.

Flag leaves of other wheat cultivars infected with the disease did not show any symptoms. A review of literature indicates that there is no report regarding occurrence of characteristic symptoms on flag leaves of wheat plants infected with loose smut.

It would be necessary to know whether the characteristic yellowing and necrosis of the flag leaf of the infected plants of Sonalika (RR-21) is race specific or this cultivar reacts in a similar fashion to all of the physiological races of the pathogen.

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### An Undescribed Anthracnose Disease of *Chlorophytum* from Maharashtra

*Chlorophytum tuberosum* Baker var. *strictum* is a beautiful tuberous herb commonly grown in gardens as an ornamental. A serious anthracnose disease was noticed on some of these plants, at Poona in the form of well defined necrotic spots which were sub-circular to angular mostly originating from tips or margins. The central portions of such necrotic lesions were ashy-white, papery, slightly sunken with clear-cut dark-brown borders, which, on microscopic examination were found to be infected by a species of *Colletotrichum*. The fungus sporulated profusely with abundant acervuli on necrotic spots within the course of 48 hours when incubated in moist chambers, and showed such morphological characters: Mycelium pale-olivaceous to brown, septate to highly branched. Acervuli spherical to globose, dark, scattered, deeply seated, covered with profuse setae, measure 85-170  $\mu$  in diam., setae abundant, stiff, long, broader at the base, uniformly tapering and pointed at the apex, septate (3-6) dark-brown, 20-45 per acervulus, majority straight and stiff but some slightly bent, 57-285  $\times$  3-4  $\mu$ ; conidia profuse, in gelatinous masses, hyaline, one-celled, falcate with pointed ends, measure 19-26.6  $\times$  2-3  $\mu$ . Based on these detailed morphological characters and dimensions of acervuli, setae and conidia the fungus under study was identified as *Colletotrichum dematium* (Fr.) Grove<sup>1,2</sup>. This species is characterised by relatively small falcate conidia and by dark often stromatic acervuli with stiff setae<sup>2</sup>. This constitutes the first known report of the fungus on the above host. The material is deposited at Ajrekar Mycological Herbarium at M.A.C.S., Poona, under No. AMH 1916.

Grateful thanks are offered to Prof. M. N. Kamat for his keen interest, to the Director for laboratory facilities and to Prof. T. S. Mahabale for his kind help in the identification of host plant.

M.A.C.S. Research Institute,  
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December 29, 1973.

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## REVIEWS AND NOTICES OF BOOKS

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**Calculus with Finite Mathematics for Social Sciences.** By Mary W. Gray. (Addison-Wesley, Massachusetts). 1972. Pp. ix + 593. Price \$12.95.

This book serves the purpose extremely well for which it is written, namely, to serve as a text-book for the students of the social, biological and management sciences. The book has application-oriented approach and introduces the topics of finite probability, continuity, differentiation and integration of functions, matrices and linear equations, finite dimensional vector spaces, linear transformations, series, elementary trigonometry, and differential and difference equations. A good number of problems are provided for practice at the end of each section and answer to selected problems are given at the end.

A welcome feature of the book is that the reader's attention is attracted towards the "warnings" spread throughout the material presented. It is necessary many a time to point out "DON'T"s along with or rather than "DO"s. This is done through these "warnings" so that the students come to know positively the common errors that they are likely to commit.

Maze of details of functions of one variable is avoided purposely to use the time to study beneficially the theory of functions of more than one variable and of partial differentiation which is so useful for those for whom the book is written and for which the time must therefore be found.

It is a very good decision by the author to present the theory of integration in its own right rather than introducing integration first as a process reverse of differentiation as is done many times. As such the relationship between area and the

concept of integration is explained and emphasized first. Then the fundamental theorem of integral calculus is introduced and finally indefinite integral is brought in. The concepts understood in this fashion will be settled and fixed with the student in a better way.

The opening example in the discussion of sequences that the next term in the sequence 2, 4, 6, 8 . . . is not unique brings home the point that one has to be alert and should not become the victim of conventional thinking. The general term for the sequence can be  $2n$  or  $n^2 - 10n^3 + 35n^2 - 48n + 24$  and hence 10 and 34 are both the candidates for the next term in the above sequence. Thus a sequence cannot be specified by writing its first few terms.

There are three small appendices to the book. First is on limits wherein the proofs of six theorems on limits that were only stated in the main text are proved. Second appendix explains nicely how to use common logarithms for computational purposes. Third appendix is devoted to explain Cramer's rule to solve a system of  $n$  linear, algebraic, non-homogeneous, consistent and independent equations in  $n$  unknowns. Four small tables on natural trigonometric functions, natural logarithms of numbers, exponential functions, and four-place logarithms of numbers are given at the end for ready reference and use by students while carrying out numerical calculations.

There is a well-prepared index available at the end of the book. Figures in the book are neatly drawn. Paper used, printing and get-up of the book is very good. It is worthwhile to bring out this book in an inexpensive edition.

V. G. TITIKAR.

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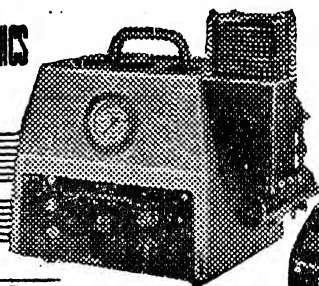
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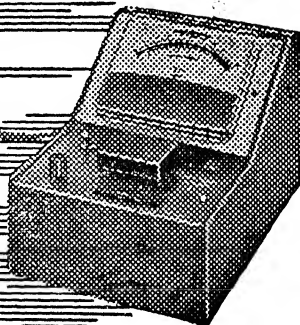
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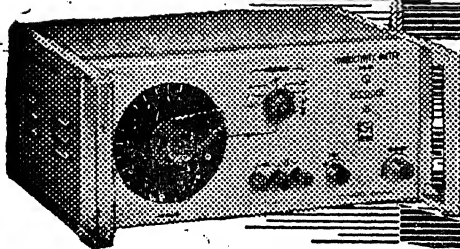
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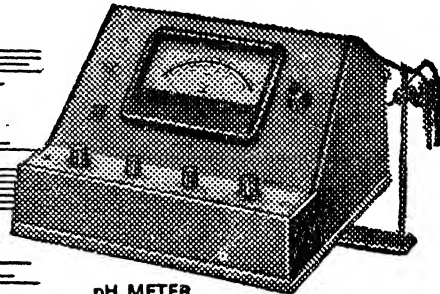
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# PROTEIN SYNTHESIS DURING EARLY GERMINATION

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**T**HE control of early developmental processes and subsequent growth is mainly dependent on the specific transcriptional and translational functions in the living organisms. Protein synthesis is considered to be the earliest molecular process in seed germination<sup>1</sup>. Regulation of the protein synthesising machinery is related to repression as well as derepression of the genome.

During germination, part of the proteins are hydrolysed to peptides and amino acids in the storage organs and translocated to the developing embryos. There seems to be a sequential appearance of various proteins in germinating seeds. There has been speculation on the time of synthesis of proteins during germination. Bhatia and Nilson<sup>2</sup> showed that proteins did not change until 48 hr in wheat seed germination; while Maller<sup>3</sup> observed changes in protein separation by 4 to 12 hr in onion. Hence it was aimed to study the process of protein synthesis in mungbean (*Phaseolus aureus* L.) and runner bean (*Phaseolus coccineus*) axes during early germination.

## MATERIALS AND METHODS

The mungbean seeds were purchased from the local market. The runner bean seeds were purchased from Supergran, Mechelen, Belgium. Uniform mungbean seeds according to size and weight were sterilized with 1% calcium hypochlorite for 2 min and washed in sterile water. The seeds were germinated in 0.01 M potassium dihydrogen phosphate pH 6.0 at 30° C in a metabolic shaker with 80 to 90 oscillations per minute. At the end of each incubation, which varied from 4 to 10 hr, the axes were separated manually, after arresting the incubation by plunging the seeds in cold water. They were immediately taken for analysis or stored at -15° C until needed.

Embryonic axes of runner bean were excised manually and stored at 4° C in a desiccator in dark until needed. The axes were germinated in dark at 30° C in a medium referred as GM (Germination Medium)<sup>4</sup> containing *tris*-HCl (pH 7.6), 0.005 M; KCl, 0.02 M; sucrose, 10 mg.ml<sup>-1</sup> and chloramphenicol, 10 µg.ml<sup>-1</sup>. The incubation was stopped by the addition of ice-cold water at the end of each interval. The axes were either used immediately or stored at -15° C until required.

*Protein Synthesis in vivo.*—Studies on initiation of protein synthesis were conducted with 10 uni-

form mungbean seeds/10 runner bean axes. The incubation medium contained 0.2 µc and 0.3 µc of <sup>14</sup>C-leucine-(U), (331 µc per µmole) for mungbean and runner bean respectively. The incubation was arrested by adding cold leucine (10<sup>-2</sup> M). The axes were then homogenized in a buffer containing *tris*-HCl (pH 7.8), 0.01 M; MgCl<sub>2</sub>, 0.01 M; and KCl, 0.02 M. The contents were centrifuged briefly and the volume made to 10 ml. Suitable aliquots were precipitated with trichloroacetic acid (TCA). The TCA-insoluble material was incubated at 80° C for 20 min, cooled and filtered on Whatman GF/C glass fibre filters. The precipitate was washed twice with 10% TCA and once with 70% ethanol. The filter was dried and counted with 5 ml of scintillation mixture (4 g PPO per litre of toluene) and radioactivity counted in a Packard (model 574) Liquid Scintillation Spectrometer. The results are expressed as cpm label incorporated into acid-insoluble material per 10 axes.

*Separation of Protein on Polyacrylamide Gels.*—The axes were homogenized in 3 or 5 ml buffer consisting of *tris*-HCl (pH 7.8), 0.01 M; magnesium acetate, 0.001 M; KCl, 0.005 M and mercapto-ethanol, 0.0015 M respectively. The homogenate was centrifuged at 165,000 g for 180 min in a SW 50 rotor. The resulting supernatant was subjected to separation of proteins on polyacrylamide gels. Before applying the sample on the gel, the protein concentration in all the samples (different hours) was adjusted to be uniform by proper dilution. This solution was uniformly mixed with 40% sucrose and an aliquot was layered on the gel and electrophoresed.

The disc electrophoresis of Davis<sup>5</sup> was adopted using 7.5% acrylamide with *tris*-glycine buffer (pH 8.3). The electrophoresis was conducted with 6 to 8 mA per gel using bromophenol blue as tracker. After electrophoresis, the gels were stained in a solution of 1% amido black in 7% acetic acid and destained electrophoretically in 7% acetic acid after an hour. The coloured bands of proteins were traced using a Varicord densitometer.

## RESULTS

In mungbean axes, protein synthesis is initiated after the 4th hour. A little decrease is observed at the 7th hour, followed by a further increase towards the 10th hour (Fig. 1). Protein synthesis in excised embryonic axes of runner bean is found



to initiate even by the first hour of imbibition and increases at later stages. The incorporation of leucine is inhibited by cycloheximide (Table I).

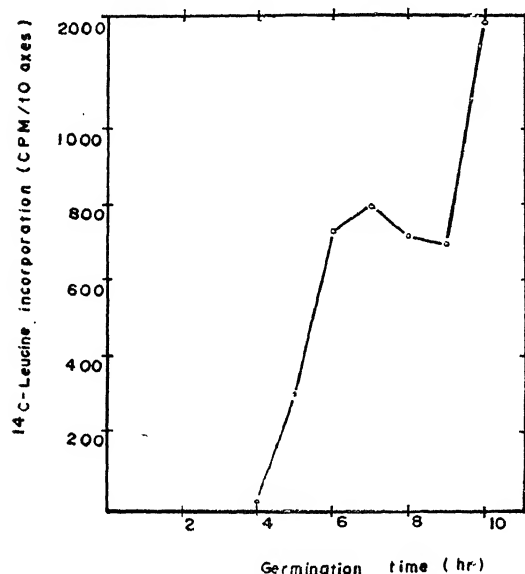


FIG. 1.

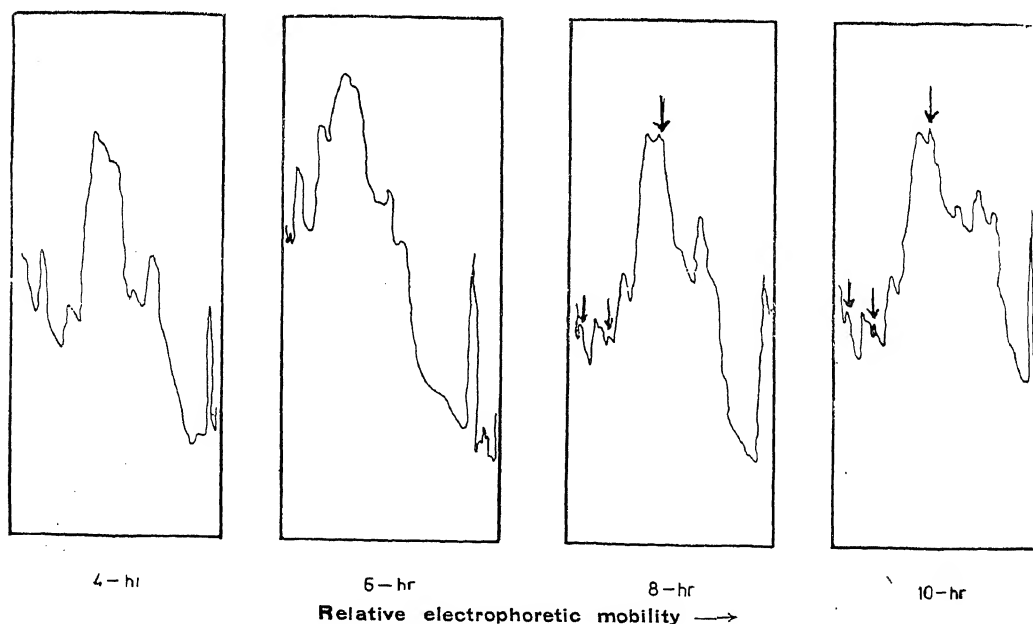


FIG. 2

The protein fraction from high-speed (165,000 g) supernatant when electrophoresed gave the changing pattern of proteins during germination and the results are indicated in the densitometer tracings

axes were also subjected to electrophoresis. The separation patterns (Fig. 3) are modified in germinated axes. The difference between dry and germinated axes is clear, as seen in the diagram,

TABLE I

Effect of cycloheximide on protein synthesis in runner bean axes

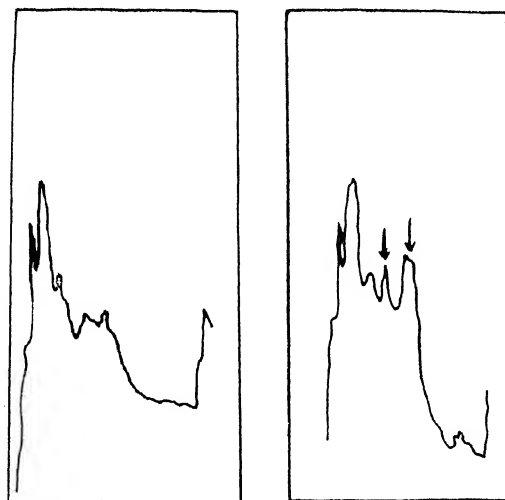
Time (hr)	normal	+ Cycloheximide (1 mg/ml)
From		
	(cpm/10 axes)	
0 to 1	543	23
5 to 6	6863	97
11 to 12	13705	85

(Fig. 2). There is a marked difference in the separation pattern of different protein bands during early germination hours in mungbean. Little change is observed in the pattern between 4 and 6 hr. By 8 and 10 hr periods, the number of bands increases. In 10 hr period, sharp tracings are obtained for almost all the bands (Fig. 2) and the newly formed bands become very conspicuous as compared to that found by 8 hr (indicated by arrows).

The supernatant fractions of runner bean obtained from ungerminated and 12 hr germinated embryonic

### DISCUSSION

There is relative increase in the synthesis of protein in both mungbean and runner bean axes as proved by  $^{14}\text{C}$ -leucine incorporation. The protein breakdown and resynthesis occur during early germination period; this is in agreement with the electrophoretic separation of proteins (Figs. 2 and 3). Protein synthesis is initiated after 4 hr in mungbean and even from the first hour in runner bean axes. Since the leucine incorporation is inhibited by cycloheximide, it is evident that *de novo* protein synthesis occurs even from the first hour of germination in runner bean. In barley seeds<sup>6</sup> also, protein synthesis starts even by the first hour of germination.



Ungerminated

12 hr germinated

Relative electrophoretic mobility →

FIG. 3

The fact that protein synthesis is observed at very early stages of germination, points out that the synthesis may be directed by a preformed messenger as reported for cotton embryos<sup>7</sup>. It is also probable that imbibition triggers the protein synthesising machinery by the formation of polysomes which are required for protein synthesis<sup>1</sup>. Levinthal *et al.*<sup>8</sup> reported that synthesis of early proteins is dependent on conserved messenger in *Bacillus subtilis* spores.

The formation of polyribosomes and the consequent activation of protein synthesising capacity, immediately after imbibition during early developmental stages in wheat embryos<sup>9</sup>, provide evidence for protein formation. Hence, it can normally be expected that during early germination, varied

changes in proteins do take place. The results presented in Figs. 2 and 3 reveal that spectra of protein are altered during germination of embryonic axes. Since the axes are growing fast and metabolically very active, synthesis of proteins may occur as evidenced by the electrophoretograms. The formation of additional bands and modified bands may be due to new synthesis. It is also probable that the storage proteins are degraded and give rise to a different protein pattern. Further, proteins present in ungerminated axes may be transformed and lead to the formation of new bands. However, in contrast to the present results, Bhatia and Nilson<sup>2</sup> reported in wheat seeds, that no difference could be observed in protein separation patterns before 48 hr of germination. In *Phaseolus vulgaris* seeds<sup>10</sup>, the number of bands of albumin disappears during early stages of germination and several new components are formed in later germination period.

It was observed that different classes of proteins synthesised could reflect the time of transcription of the corresponding portions of the genome. Though there appears to be a definite variation in protein separation patterns, identification of individual protein components is, at best, very uncertain. In addition, in the absence of the genetic data, the appearance and disappearance of particular bands cannot be ascribed to gene function which modifies secondarily in each tissue to meet specialized requirements for growth and development, as reported by Bhatia and Nilson<sup>2</sup>.

### ACKNOWLEDGEMENT

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## SUSCEPTIBILITY OF *Aedes novalbopictus* CELL LINE TO INFECTION WITH SOME ARBOVIRUSES

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### ABSTRACT

Susceptibility of *Aedes novalbopictus* cell line to infection with chikungunya (Group-A); West Nile, Japanese encephalitis, dengue types 1, 2, 3 and 4 (Group-B); Chandipura (VSV group) and Ganjam (ungrouped) viruses has been studied. All the above-mentioned 9 arboviruses multiplied without showing any obvious cytopathic effect in this cell line. Detailed studies carried out on the growth of 4 types of dengue viruses indicated that the titre of cell associated virus was higher than that of extracellular virus.

### INTRODUCTION

**E**STABLISHMENT of several new cell lines from many species of mosquitoes and their susceptibility to infection with some common arboviruses have been reported from this laboratory earlier<sup>1-14</sup>. Recently, new cell lines were established from yet another species of mosquito, viz., *Aedes novalbopictus*<sup>15</sup>. The present communication deals with the studies carried out on the susceptibility of this new cell line to infection with some arboviruses.

### MATERIALS AND METHODS

**Cell line.**—Cells from a continuous line of *A. novalbopictus* (ATC-173) from 41 and 45 passage levels were employed. The details of the maintenance of the cell line and the culture medium were described earlier<sup>15</sup>. *A. albopictus* cells<sup>1</sup> from the line ATC-15 from 19 to 30 passage levels were employed to assay some of the viruses.

**Viruses.**—The following 9 common arboviruses representing the major serogroups were tested.

**Group A:** Chikungunya (CHIK), VRC No. 634029, mouse passage 12.

**Group B:** West Nile (WN), VRC No. G 22886, mouse passage 17.

Japanese encephalitis (JE), VRC No. P 20778, mouse passage 10.

Dengue type 1 (DEN-1), VRC No. 703311, 6 passages in *Aedes albopictus* cell culture.

Dengue type 2 (DEN-2), VRC No. 68883, 4 passages in *Aedes albopictus* cell culture.

Dengue type 3 (DEN-3), VRC No. 703539, 6 passages in *Aedes albopictus* cell culture.

Dengue type 4 (DEN-4), VRC No. 684996, 4 passages in *Aedes albopictus* cell culture.

**VSV Group:** Chandipura (CHP), VRC No. 653514, mouse passage 20.

**Ungrouped:** Ganjam (GAN), VRC No. G 619, mouse passage 5.

**Virus inoculation.**—The techniques employed to study the multiplication of the viruses were essentially the same as described by Singh and Paul<sup>2</sup>. Briefly, batches of 20 monolayer culture tubes were inoculated with 0.1 ml of virus suspension as to give 3 to 4 dex TCID<sub>50</sub> or LD<sub>50</sub> of the virus per culture tube. The inoculum were simultaneously titrated either in mice or in tissue culture as to determine the exact dose of virus inoculated. After 2 hours absorption, infected cell sheets were washed twice with Rinaldini's salt solution and fed with fresh medium. In order to study the growth of 4 types of dengue viruses in detail, extracellular and cell associated viruses were harvested separately from 2 infected culture tubes at '0' hour and on post-inoculation (PI) days 3, 6, 10 and 15. Whereas, in case of CHIK, WN, JE, CHP and GAN viruses, they were harvested only on the 10th PI day just to test whether *A. novalbopictus* cells supported their multiplication. Batches of tubes containing 0.5 ml medium without cells, inoculated with 0.1 ml virus suspension, were used as controls.

**Virus assay.**—All the four types of dengue viruses were assayed in normal *A. albopictus* cell culture. Whereas, other viruses were assayed in infant (2 to 3-day-old) mice by intracerebral route. The titres were expressed as dex TCID<sub>50</sub> for tissue culture or LD<sub>50</sub> for mice. Identity of the viruses from the harvested culture fluids was confirmed serologically in complement fixation test.

### RESULTS

The results indicated that all the nine arboviruses tested multiplied in *A. novalbopictus* cells (Table I), without showing any obvious cytopathic effect (CPE). Approximately 100 to 100,000 fold increase in the virus concentration was observed during the first 10 days with these viruses.

The growth curve studies with 4 types of dengue viruses in *A. novalbopictus* cells (Fig. 1) indicated

that the concentration of cell associated virus was higher than the virus in the extracellular fluid. The difference was generally 2 dex or less. Among the 4 types of dengue virus, type 2 showed approximately 10,000 fold increase, whereas, the others showed approximately 1,000 fold increase during the 15 days of observation.

10,000 fold increase, whereas, CHP showed 1,000 fold increase.

# DISCUSSION

The multiplication of nine arboviruses in *A. novalbopictus* cells was comparable to that in *A. albopictus* cells as studied earlier<sup>2,3</sup>. While

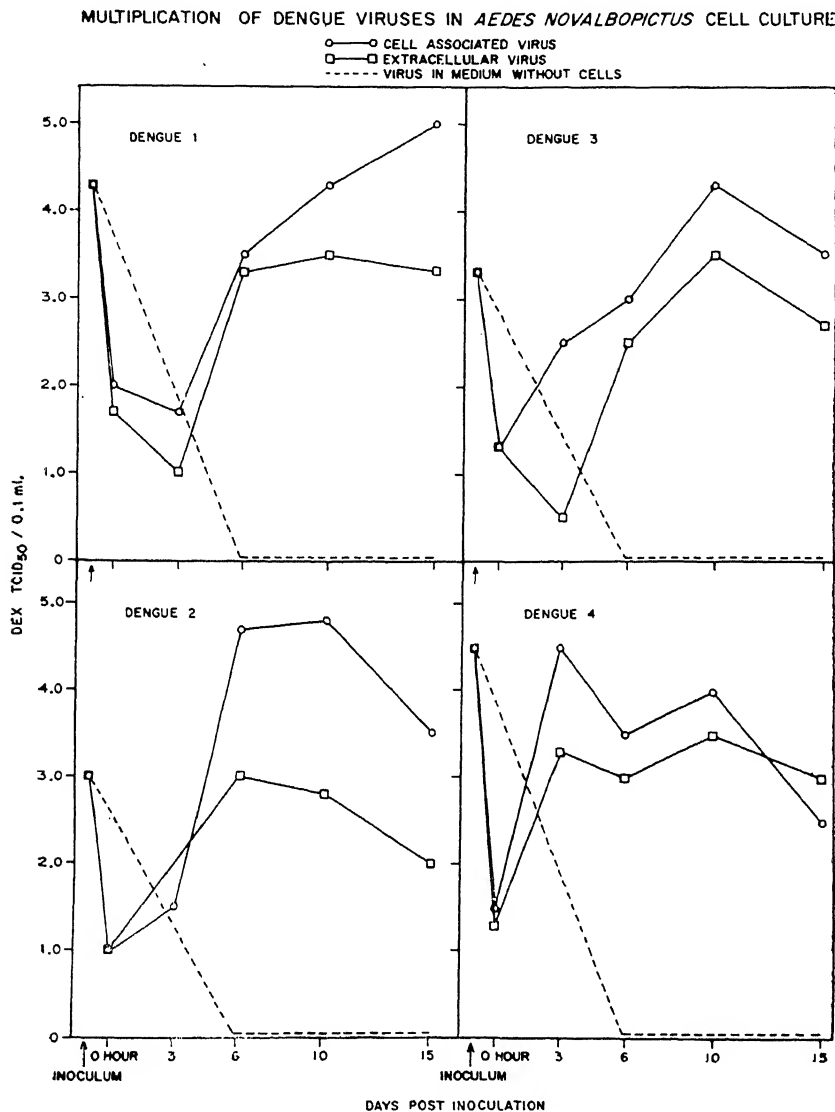


FIG. 1.

Among other viruses, when tested on the 10th PI day WN virus showed the maximum increase (approximately 100,000 fold), whereas, JE virus showed the minimum increase (approximately 100 fold), CHIK and GAN viruses showed approximately

*A. albopictus* cells showed CPE with group B mosquito borne arboviruses, viz., WN, JE, DEN-1, 2, 3 and 4, no obvious CPE was detected in *A. novalbopictus* cells. Studies carried out on the susceptibility of *A. albopictus* cells to infection with

TABLE I

Multiplication of some arboviruses in *Aedes novalbopictus* cell line

Virus	Titre					
	Ino- culum	0 Hour	PI Days			
			3	6	10	15
1. Chikungunya*	2.5	NT	NT	NT	6.5	NT
2. West Nile*	1.5	NT	NT	NT	6.0	NT
3. Japanese en- cephalitis*	2.5	NT	NT	NT	4.0	NT
4. Dengue type 1**	4.3	2.0	1.7	3.5	4.3	5.0
5. Dengue type 2**	3.0	1.0	1.5	4.7	4.8	3.5
6. Dengue type 3**	3.3	1.0	2.5	3.0	4.3	3.5
7. Dengue type 4**	4.5	1.5	4.5	3.5	4.1	3.1
8. Chandipura*	3.5	NT	NT	NT	6.5	NT
9. Ganjam*	2.5	NT	NT	NT	6.5	NT

\* Virus titre, dex LD<sub>50</sub>/0.02 ml.

\*\*Virus titre, dex TCID<sub>50</sub>/0.1 ml, cell associated virus.  
NT=Not tested.

4 types of dengue viruses (Guru and Bhat, unpublished data) indicated that yield of these viruses was higher in *A. albopictus* cells than in *A. novalbopictus* cells. It is thus evident that *A. albopictus* cells are more susceptible to arbovirus infection than *A. novalbopictus* cells.

It is interesting to note that in *A. novalbopictus* cells infected with 4 types of dengue viruses, the concentration of cell associated virus was always higher than that present in the extracellular fluid. Similar results have been obtained with these viruses in *A. albopictus* cells (S. N. Ghosh, personal communication).

Earlier studies carried out in this laboratory on the susceptibility of some of the mosquito cell lines

to infection with arboviruses indicated that *A. aegypti* cells supported the multiplication of 3 out of 9<sup>2-3</sup>, *A. w-albus* 3 out of 6<sup>11</sup>, and *A. vittatus* 4 out of 6<sup>13</sup> viruses tested. Thus it is evident that *A. novalbopictus* cells are more susceptible to arbovirus infection than *A. aegypti*, *A. w-albus* and *A. vittatus* cells.

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# RECORD FISH PRODUCTION WITH INTENSIVE CULTURE OF INDIAN AND EXOTIC CARPS

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**F**ISH culture has a long history in India and the association of species of Indian major carps in pond fish culture has been known to the fish farmer. The productions, however, have been low because food niches of the pond remained under-exploited, and dependence was mostly on the natural fertility of the ponds to produce fish food. With the introduction at Cuttack of exotic species of fishes, the Chinese silver carp and grass carp and the common carp of Indonesian strain by the Pond Culture Sub-station of the Central Inland Fisheries Research Institute, true multi-species culture of the three exotic carps and indigenous major carps were undertaken in experimental ponds. Experience gained through experimentation led to the gradual increase of fish production in still-waters and a production as high as 7,500 kg/ha/annum has been recently obtained which is reported in the present communication. The production was achieved by intensive multi-species culture of fish employing a high stocking density in desirable proportion supported by judicious application of fertilizers and supplementary feeding. The highest production (net) recorded from India earlier to this was 6,286.1 kg/ha/yr (Jhingran, unpublished). Bardach *et al.* (1972) mentioned that under favourable circumstances productions of 7,500 to 8,000 kg/ha may be attained by polyculture in the Far East. Yashouv and Halevy (1972) have reported of productions as high as 10,620 kg/ha/yr with poly-culture of tilapia, common carp and silver carp. Chaudhuri (FAO, 1971) achieved fish production of 10,390 kg/ha in a year in Burma by culturing the Indian major carps together with small numbers of carp hybrids and gourami.

The experiments reported herein were undertaken in two ponds each 0.25 ha in area which resulted from renovation of a section of the moat of the Killa Experimental Fish Farm at Cuttack. This section had a poor record of fish production as the water sheet was rendered unsuitable for fish culture due to accumulation of coaltar flowing into the water from drums dumped nearby.

The ponds were poisoned with mahua oil cake applied @ 250 ppm which was followed by application of quicklime @ 100 kg/ha. Because of entry of some murels from nearby sources the pond 6B had to be poisoned a second time after a month of the initial poisoning. Hereafter, the fingerlings for culture were released.

The ponds were stocked in September, 1972 with 2-3 month old fingerlings raised from fry of induced bred fish. The stocking density used for both the ponds was 10,540 fingerlings per hectare of the cultivated cyprinids which included the Indian major carps catla, rohu, mrigal, the Chinese silver carp and grass carp and the common carp stocked in the ratio of 1:3, 1:2, 1:2 respectively. To this was added 544/ha of miscellaneous fish in one and in the other 568/ha. The former pond has been denoted as 6A and the latter as 6B. Species comprising the miscellaneous category included *Notopterus chitala*, *Ompok bimaculatus*, *Mystus seenghala* and *Pangasius pangasius*. The purpose of introduction of these species was to further increase production by utilising the insects, shrimps, molluscs and weed fishes (inadvertent entry) which are not made use of by the cultivated carps but compete with them for food.

Fertilization was effected by means of inorganic (a mixture of urea and triple superphosphate) and organic (cowdung) fertilizers, the former applied once a month and the latter once a quarter. Fertilization was stopped when algal blooms persisted and fish appeared to be in distress particularly during early hours of the morning. The quantum of inorganic fertilizer used was 1,530 kg/ha/yr for 6A and 1,140 kg/ha/yr in 6B. Each of the ponds received cowdung @ 14,400 kg/ha/yr. The stocked fish were daily fed with artificial feed, which was a mixture of groundnut oil cake and rice bran, a fortnight onwards after stocking. The quantity of feed put in pond 6A was 12,852 kg/ha/yr and 13,340 kg/ha/yr in 6B. Feeding was suspended or reduced during periods of low water level in the ponds or when the ponds were having a thick bloom of *Microcystis*.

Feeding was undertaken by broadcasting the feed from the pond margin during the first half of the rearing period whereafter the feed was made into a dough and a number of balls of this material was placed in trays hung at different depths in the pond. Chopped bits of *Enhydra fluctuans* were mixed with the feed in the latter half of the culture period. Grass carp were given weeds particularly *Spirodela*, *Najas* and *Hydrilla* regularly.

Periodical removal of fish which had reached the marketable size of 1 kg was carried out in both the ponds and the remaining finally harvested after one year of culture. The earliest to be harvested

TABLE I  
Average weights of fishes as computed from the total numbers recovered and total weights realised of each species

Species	POND 6 A			POND 6 B		
	Average weight in gm	Survival (%)	Contribution by weight (%)	Average weight in gm	Survival (%)	Contribution by weight (%)
Silver carp	.. 1,152	30.85	13.67	1,032	65.90	19.04
Catla	.. 1,197	67.92	15.00	1,179	71.69	11.94
Rohu	.. 754	94.93	39.42	854	97.72	35.14
Grass carp 1st lot	.. 1,106	76.60	15.63	1,219	68.68	11.84
Grass carp 2nd lot	.. ..	..	..	1,542	89.00	7.30
Mrigal	.. 766	94.33	13.39	654	100.00	9.54
Common carp 1st lot	.. 1,516	0.41	2.30	1,772	1.17	0.86
Common carp 2nd lot	.. ..	..	..	977	92.59	2.61
<i>Ompok</i>	.. 79	32.00	0.07	102	60.00	0.01
<i>Chitala</i>	.. 163	80.00	0.83	238	91.91	1.17
<i>Mystus</i>	. 330	36.36	0.12	398	75.00	0.02
<i>Pangas</i>	.. 733	60.00	0.14	742	100.00	0.03

were some grass carp and silver carp and a few common carp from both the ponds at the end of 5 months' rearing. Some catla could be harvested after 6 months. A second lot of 100 nos. of grass carp and 54 nos. of common carp were introduced in pond 6 B, in which fish were found to be growing better. The grass carp were introduced with the idea of raising a second crop of the species, which was realised; whereas the common carp were put as it was observed from the periodic samplings that the earlier introduced numbers of the species had disappeared either through mortality or poaching.

The experiments were concluded after one year when the fish were finally harvested. Table I denotes the average weights attained by the fish in the two ponds along with their survival percentages and also the contribution of each species as percentages of the total yield. The high survival despite the high stocking density obtained with rohu puts this species in the first place as regards species-wise contribution to the total gross production which in the case of pond 6A is 2,261.6 kg/ha and for 6B 2,638.6 kg/ha. The species that belied all expectations was the common carp which were almost absent from the fish harvested.

The gross and net productions recorded in pond

6B was 7,500 kg/ha/yr and 7,343 kg/ha/yr respectively and that in 6A 5,734 kg/ha/yr and 5,652 kg/ha/yr respectively and is the highest recorded yield in pond culture operations in India. The former figure can be considered as outstanding for a pond not having facilities for recirculation of water. This could truly be considered as intensive fish culture since the stocking density used was more than double the rate ordinarily followed in composite fish culture in India.

#### ACKNOWLEDGEMENT

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## LETTERS TO THE EDITOR

### FERROMAGNETISM IN INSULATING $\text{Er}_2(\text{WO}_4)_3$

MAGNETIC properties of lanthanide (rare-earths) compounds have been a subject of considerable interest in recent years<sup>1,2</sup>. Many of their compounds particularly orthoferrites<sup>3</sup> are relatively well studied. Several insulating compounds with ferromagnetic interaction like  $\text{EuO}$ ,  $\text{EuH}_2$ ,  $\text{EuSe}$ ,  $\text{ErCrO}_3$ ,  $\text{ErFeO}_3$ ,  $\text{YCrO}_3$ , etc., have also been reported. We are studying the electrical and magnetic properties of their tungstates<sup>4,5</sup> and this note reports our study regarding the temperature variation of magnetic susceptibility of powdered sample of  $\text{Er}_2(\text{WO}_4)_3$  and it has been found to be a ferromagnetic.

$\text{Er}_2(\text{WO}_4)_3$  sample were obtained from Chempure (India). They did not quote any specific impurity content and have claimed a purity of 99.99%. The powdered specimen is light pink in colour. Magnetic susceptibility of powdered specimen has been measured using tapered pole pieces in 4" electromagnet (Polytronic, India) and a sensitive ( $10^{-5}$  gm) non-magnetic projection type balance (Type-K-11, Keroy, India) employing standard Faraday's Method.

The results are shown in Fig. 1. From the nature of  $1/\chi_m$  vs  $T$  plot the material seems to be a ferromagnetic, with Curie-Weiss law well obeyed in the temperature range  $450^\circ\text{K}$  to  $900^\circ\text{K}$ . The different constants<sup>6</sup> have been evaluated using the straight part of the curve (Fig. 1) and its extrapolation and the values obtained are: Curie Constant ( $C$ ) =  $dT/d(1/\chi_m) = 11.43 \times 10^{-3} \text{ }^\circ\text{K/gm}$ , Paramagnetic Curie temperature ( $\theta_c$ )  $\simeq 160^\circ\text{K}$ , Molecular field parameter ( $\gamma$ ) =  $\theta_c/C = 1.4 \times 10^4 \text{ gm}$  and effective number of Bohr magneton for the lone paramagnetic  $\text{Er}^{3+}$  ion ( $p_{\text{eff}} = 3 kC/n\beta^2$ , where  $K$  is Boltzmann constant;  $n$  the number of paramagnetic ion per unit volume and  $\beta$  the Bohr magneton) = 7.43. The ground state of  $\text{Er}^{3+}$  ion is  $4\text{I}_{15/2}$ . Thus for  $\text{Er}^{3+}$  ion  $S = 3/2$ ,  $L = 6$  and  $J = 15/2$ ; which yields the value of Lande's splitting factor ( $g$ ) = 1.2 and the value of  $p_{\text{eff}} = g \sqrt{J(J+1)}$  for the ion to be 9.585. There is marked discrepancy in the experimental and theoretical value of  $p_{\text{eff}}$ . It is not surprising. Usually<sup>1</sup> a low value of  $p_{\text{eff}}$  for lanthanide ion in rare-earth solids are obtained. The principal reason being the hybridization of higher orbit electrons with  $4f$  electrons at higher temperature and to some extent the surrounding crystal field. An increase in the number of  $4f$  electrons will decrease the actual and hence experimental value of  $p_{\text{eff}}$ .

A discrepancy and upward trend in  $1/\chi_m$  vs  $T$  curve little above  $\theta_c$  is usual trend of many ferromagnetic solids but a slight changed nature round  $350^\circ\text{K}$  in the curve (Fig. 1) needs detailed investigation. There might be a change in the magnetic structure of the solid round this temperature. In rare-earth solids  $4f$  electrons remain localized and normally do not take part in conduction<sup>1</sup>. Overall our resistivity measurement (to be published) indicate that  $\text{Er}_2(\text{WO}_4)_3$  is an insulating material with the resistivity values of the order of  $10^9 \text{ ohm-cm}$ . Thus a direct exchange between the  $\text{Er}^{3+}$  ions with its neighbouring ion is completely ruled out. Anderson type super exchange<sup>7</sup> might be the right type of interaction between the various  $\text{Er}^{3+}$  ions. The crystal structure of  $\text{Er}_2(\text{WO}_4)_3$  have been reported few years back<sup>8</sup>. It is easy to get an estimate of average exchange energy parameter ( $J_e = ng^2\beta^2/27$ , where  $Z$  is the number of nearest neighbours). Taking six nearest neighbours, one gets  $J_e = 8.88 \times 10^{-5} \text{ eV} \simeq 1.055^\circ\text{K}$ . However presence of oxygen shall play the important role. A better understanding of  $\text{Er}-\text{O}-\text{W}-\text{O}-\text{Er}$  ion needs low temperature study of magnetization and magnetic structure of the compound.

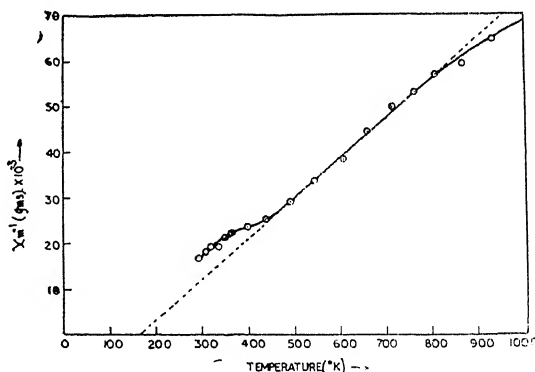


FIG. 1. The variation of the inverse of magnetic susceptibility ( $1/\chi_m \text{ gm}$ ) with temperature ( $T^\circ\text{K}$ ) for the powdered specimen of  $\text{Er}_2(\text{WO}_4)_3$ .

A slight discrepancy in  $\chi_m$  vs  $T$  curve above  $900^\circ\text{K}$  (Fig. 1) from the Curie-Weiss law might be due to positive contribution to  $\chi_m$  coming from the paramagnetic contribution of conduction electrons whose number will be sufficient at this temperature.

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### 1-(2-PYRIDYLAZO)-2-PHENANTHROL (PAP) AS A VISUAL INDICATOR IN EDTA-TITRATIONS

1-(2-PYRIDYLAZO)-2-Phenanthrol (PAP), first prepared by Chiswell *et al.*<sup>1</sup>, has been used earlier as a visual indicator in the determination of cobalt (II)<sup>2</sup> and copper(II)<sup>3</sup> by direct titration with EDTA. The present communication describes the microdetermination of nickel(II), zinc(II), cadmium (II) and lead(II) by back titrating the excess of EDTA against copper(II). Direct titrations of these metal ions are not possible due to comparable stability of metal-indicator and metal-EDTA complexes in the case of nickel(II) and due to dull colour changes from metallised to free indicator in other cases.

The pH ranges found suitable for titrating the various metal ions are: Ni(II) (3.5–6.6), Zn(II) (4.00–7.1), Cd(II) (4.9–5.8), Pb(II) (5.5–7.0). The temperature range suitable for the determinations is 15°–60° C. Accurate results are obtained, using copper(II) solutions of concentrations 0.01–0.001 M, for back titration. With more concentrated solutions, the deep blue colour of copper(II)-EDTA complex obscures the end point, while with too low a concentration of copper(II), faint colour of the copper-PAP complex does not give a sharp end point. Four drops of indicator solution (0.002 M) in dioxan are sufficient to cause a sharp and instantaneous colour change (green to red-violet) at the end point. The limits up to which various anions are tolerated are given in Table I.

Several metal ions, *e.g.*, Ti(IV), V(V), Fe(II) and Fe(III), Hg(II), Al(III) and Cd(II) were found to interfere. However, interference due to

TABLE I

Tolerance limits for anions (in mg) 5 ml of 0.01 M metal ion solution, diluted to 30 ml

Anion	Ni <sup>2+</sup>	Zn <sup>2+</sup>	Cd <sup>2+</sup>	Pb <sup>2+</sup>
Citrate	50	5	30	20
Tartrate	150	500	200	10
Oxalate	2	2	5	10
Sulphocyanide	500	400	500	50
Sulphite	500	400	400	300
Fluoride	500	500	500	500
Chloride	500	400	400	400
Bromide	400	500	500	500
Phosphate	500	400	300	500
Borate	300	200	20	50
Thiosulphate	5	Interferes	interferes	interferes
Thiourea	10	10	10	10
Nitrite	400	300	400	500
Iodide	100	50	100	100

5 mg each of Hg(II) and Cd(II) in the case of Ni(II), and Pb(II) could be avoided by masking with iodide and Al(III) in all the cases by fluoride. Any amount of the alkaline earth-metals and rare earths may be tolerated.

**Recommended Procedure.**— Pipette out 5.0 ml of 0.01 M metal ion solution in a 100 ml titration flask and add a known excess (9.0 ml) of 0.01 M EDTA solution. Buffer to a suitable pH [Ni(II), 4.99; Zn(II), 6.5; Cd(II), 4.99; Pb(II), 6.2] by sodium acetate/acetic acid buffer, add four drops of the indicator solution (0.002 M) in dioxan and 5 ml of methanol. Titrate slowly against standard M/100 copper sulphate solution to a colour change from light green to red-violet.

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**N-BENZOYL-PHENYLHYDROXYLAMINE  
AS A GRAVIMETRIC REAGENT FOR ZINC**

N-BENZOYL-phenylhydroxylamine (N-BPHA) has been used in the gravimetric and/or spectrophotometric determination of various metals<sup>1</sup>. In our present investigation, N-BPHA has been found to be a suitable chelating agent for gravimetric determination of zinc. The reagent reacts quantitatively with zinc (II) in the pH range 5.3 to 6.8, giving an insoluble metal complex. The white zinc complex is thermally stable up to 251° C and can be directly weighed as  $\text{Zn}(\text{C}_{13}\text{H}_{10}\text{O}_2\text{N})_2$  after drying at 110–120° C. Interferences due to several ions, e.g., Ca (II), Sr (II), Ba (II), Mg (II), In (III), Be (II), Hg (II), Cd (II), As (III) Sb (III), Mo (VI), W (VI), La (III), U (VI) have been avoided by working at controlled pH and by using suitable masking agents. The accuracy is better than  $\pm 0.4\%$ .

The reagent was prepared by the method reported earlier<sup>2,3</sup>. Reagent solutions were prepared by dissolving the required amounts in 10–20 ml of 90% ethanol before use. A standard solution of zinc (II) was prepared by dissolving zinc chloride in 0.1 N hydrochloric acid. The metal content was determined gravimetrically<sup>4</sup>. All other solutions were prepared from reagent grade chemicals. A Cambridge pH meter was used for pH measurements. A Chevenard thermobalance (Type 3 Adamel, Paris) was used for thermogravimetry.

**Procedure.**—To an aliquot of the standard solution of zinc (II), sodium potassium tartrate (2.0 g) solution was added and diluted to 200 ml. The pH of the mixture was then adjusted to 5.3–6.5 by dilute solutions of sodium hydroxide and hydrochloric acid. It was then heated to about 40° C and an excess (three-fold) ethanolic solution of the reagent was added with stirring. The white precipitate formed was digested on a hot water-bath with occasional stirring until the supernatant liquid became clear. The precipitate was filtered hot through a weighed sintered glass crucible of medium porosity, washed with hot water, dried at 110–120° C to constant weight (~1.5 hours), and finally weighed as  $\text{Zn}(\text{C}_{13}\text{H}_{10}\text{O}_2\text{N})_2$ . The gravimetric factor (zinc/zinc complex) is 0.1325. The results of these determinations are given in Table I.

**Determination of zinc in presence of foreign ions.**—The effect of a number of diverse foreign ions on the precipitation of zinc (II) was investigated. The results indicated that Ca (II), Sr (II) or Ba (II) had no influence on the precipitation. Separation of zinc (II), (8.81 mg) from about 5 times of Mg (II), As (III), Sb (III), Mo (VI), or W (VI) was possible following the procedure as described above. However, ammonium fluoride

(1.0 g) was used to mask the effect of about 2 times of beryllium (II) or indium (III), while potassium iodide (2.0 g) was required to eliminate the effect of about 1–2 times of cadmium (II) or mercury (II). For masking about 5 times each of lanthanum (III) and uranium (VI), ammonium carbonate (2.0 g) and ammonium acetate (2.0 g) were used, respectively. The pH was maintained around 5.6 while effecting all the above determinations.

TABLE I  
*Determination of zinc with N-BPHA*

Metal taken, mg	Metal found, mg	Error (%)
5.49	5.48	-0.18
	5.47	-0.36
12.52	12.49	-0.23
	12.50	-0.15
20.35	20.40	+0.24
	20.32	-0.14
32.20	32.10	-0.31
	32.25	+0.15

**Results and Discussion.**—The zinc (II)-N-benzoyl-phenylhydroxylamine complex melted with decomposition at  $258 \pm 1^\circ \text{C}$ . Thermolysis curve of the zinc complex showed that it was stable up to a temperature of 251° C and at a still higher temperature it decomposed with loss of weight to zinc oxide. The complex was insoluble in hot (90° C) water and sparingly soluble in ethanol, methanol, acetone, ether, benzene, chloroform and carbon-tetrachloride. The complex was analysed for zinc by igniting a weighed quantity to ZnO and for nitrogen by Dumas method. The results (Found: Zn, 13.23; N, 5.98. Calc: Zn, 13.25; N, 5.92%) were in good agreement with the formula assigned,  $\text{Zn}(\text{C}_{13}\text{H}_{10}\text{O}_2\text{N})_2$ .

**Effect of pH and reagent concentration.**—A set of experiments was carried out under stated conditions but with variation of the pH. It was observed that precipitation of zinc complex commenced at pH 4.3 and was quantitative between pH 5.3 and 6.8. In another series of experiments the pH was fixed at 5.6 and the amount of the reagent was varied. It was found that at least 2.5–3 times the theoretical quantity of reagent was necessary for complete precipitation.

**Interferences.**—Tartaric acid did not interfere with the determination of zinc. However, the precipitation was partially inhibited by oxalic acid, citric acid and completely so by disodium-EDTA and thioglycolic acid.

**Precision and Accuracy.**—The average error for several determinations of 5–32 mg of zinc (II) in a volume of 200 ml was found to be better than  $\pm 0.4\%$ .

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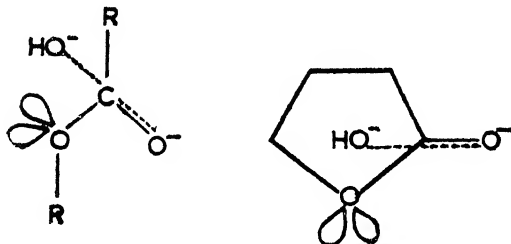
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#### CONCERNING SOLVENT EFFECTS ON THE REACTIVITY OF LACTONES TOWARDS ALKALINE HYDROLYSIS

THE enhanced reactivity of lactones over the corresponding open chain esters towards alkaline hydrolysis was attributed to the absence of repulsion between the attacking hydroxide ion and the 'lone pair' dipole on the cyclic oxygen atom because of the rearside attack of the  $\text{OH}^-$  ion on the carbonyl group which is not possible in the corresponding open chain esters<sup>1</sup>. This was schematically represented as follows:



This mechanism of rearside attack was, however, questioned by Bender<sup>2</sup> according to whom the best approximation to the transition state is the one in which the attacking  $\text{OH}^-$  ion is perpendicular to the carbonyl bond. Bender added that the results of Hall and co-workers could be explained on the

basis of this perpendicular attack still invoking a difference in electrostatic repulsion between the attacking hydroxide ion and the lone pair dipole. He could not, however, rule out the possibility of the repulsion being responsible for the increased rates of hydrolysis of lactones over that of the corresponding open chain esters. Further studies on basic hydrolysis of lactones also could not exclude this possibility<sup>3</sup>.

We have now employed solvent effects as a diagnostic tool in testing out the above hypothesis. To our knowledge, this is the first report on the differential effects of solvents on lactone saponification.

The present study concerns the alkaline hydrolysis of some  $\gamma$ -lactones, aliphatic and aromatic, and their open chain analogues. The kinetics of these reactions were studied in solvent mixtures of DMSO-water, ethanol-water and acetone-water. In Table I are presented the kinetic data in the form of  $k_{\text{lactone}}/k_{\text{ester}}$  values for the various pairs and in various solvent mixtures.

TABLE I

$k_{\text{lactone}}/k_{\text{open chain ester}}$  for the following pairs at  $30^\circ\text{C}$

	(I) $\gamma$ -butyrolactone and ethyl acetate (II) $\gamma$ -valerolactone and isopropyl acetate (III) phthalide and methyl benzoate (IV) 3-phenyl phthalide and benzyl benzoate			
Solvent (v/v)	(I)	(II)	(III)	(IV)
60% DMSO	7	20	7	..
70% DMSO	7	22	5	..
80% DMSO	11	22	6	..
60% EtOH	15	26	17	..
70% EtOH	15	32	19	..
80% EtOH	15	30	26	27
70% Acetone	11	..	7.5	..
80% Acetone	13	..	8.0	..

It is a well established fact that the  $\text{OH}^-$  ion is poorly solvated in aqueous DMSO and consequently very active in these solvent systems<sup>4</sup>. So the repulsion between the attacking  $\text{OH}^-$  ion and the 'lone pair dipole' on oxygen should be greater in aqueous DMSO. This would lead to considerable diminution in the rate of hydrolysis of open chain esters and as a consequence the ratio  $k_{\text{lactone}}/k_{\text{ester}}$  should have a greater value in aqueous DMSO relative to aqueous ethanol. Such a situation exists

indeed in the alkaline hydrolysis of dicarboxylic esters, where the ratio  $k_1/k_{11}$  increases on transfer from aqueous ethanol to aqueous DMSO<sup>5</sup> ( $k_1$  and  $k_{11}$  being the rate constants for the first and second steps of saponification of the dicarboxylic ester). This was attributed to the repulsion between a poorly solvated  $\text{OH}^-$  ion and the half ester anion leading to a considerable drop in the value of  $k_{11}$  in aqueous DMSO. What is observed in the present studies is a decrease in the ratio  $k_{\text{lactone}}/k_{\text{ester}}$  instead of an increase on solvent change from aqueous ethanol to aqueous DMSO.

Further perusal of the data also shows that even in a solvent system, variation in the content of one of the components does not produce any significant change in the ratio. Thus the rate ratio for the pair  $\gamma$ -butyrolactone and ethyl acetate has the same value of 15 in 60% EtOH, 70% EtOH and 80% EtOH. If the repulsion had been an important factor, one should have noticed a decrease in the ratio of  $k_{\text{lactone}}/k_{\text{ester}}$  with decrease in the dielectric constant of the medium as was observed in the case of dicarboxylic ester hydrolysis<sup>6,7</sup>. The solvent effects thus prove that the repulsion between the attacking  $\text{OH}^-$  ion and the 'lone pair dipole' is of minor importance in controlling the rates of hydrolysis of lactones.

We attribute the increased reactivity of lactones over that of the corresponding open chain esters to the increased polarity of the lactone carbonyl resulting from the 'cisoid' arrangement of the lone pair on etherial oxygen and the carbonyl group<sup>8,9</sup>, since in the  $\text{B}_{\text{AC}}^{2-}$  ester hydrolysis, the rate determining step is the attack of the  $\text{OH}^-$  ion on the ester carbonyl group. These structural features contribute to a greater localisation of the negative charge on the carbonyl oxygen in the transition state for lactone hydrolysis (relative to the open esters), a situation favoured by aqueous ethanol. The situation is reversed with respect to the open esters, the resultant transition state structures being stabilised by the aprotic solvents DMSO and acetone. Thus one observes a drop in the rate ratio on solvent transfer from aqueous ethanol to aqueous DMSO or aqueous acetone.

If the repulsion had been important, then one would not have observed a drop in the ratio for the pair phthalide and methyl benzoate from 26 to 8 and from 27 to 5 for the pair 3-phenyl phthalide and benzyl benzoate on solvent transfer from 80% ethanol to 80% acetone. It will be pertinent to point out here that the  $k_1/k_{11}$  ratio for the dicarboxylic ester hydrolysis does not vary much when the solvent is changed from aqueous ethanol to aqueous acetone<sup>6,7</sup>.

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#### USE OF $\text{NH}_4\text{F}$ AS A MASKENT IN THE COMPLEXOMETRIC DETERMINATION OF SOME DIVALENT CATIONS IN PRESENCE OF CALCIUM

THE need for a very rapid and accurate method for the estimation of some divalent cations in presence of calcium was felt necessary for a study of the kinetics of  $\text{Ca}^{2+} \rightarrow \text{M}^{2+}$  (where  $\text{M} = \text{Pb}$  or  $\text{Zn}$  or  $\text{Cd}$  or  $\text{Hg}$  or  $\text{Fe}$ ) exchange reactions on hydroxylapatite ( $\text{CaHA}$ ),  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , an important inorganic component of human skeletal system. The novel feature of this method over other previous methods<sup>1-3</sup> was that without the actual separation of the cations from one another from the sample solution it was possible to determine their contents complexometrically by using  $\text{NH}_4\text{F}$  as a maskent for calcium.

**Procedure.**—The Chemicals used were of AR (BDH) grade and the sample solutions were prepared in double distilled water tested for the absence of  $\text{Ca}^{2+}$  and preserved in polyethylene bottles. Since the presence of phosphate interferes with the determination of a mixture of calcium and a divalent cation mentioned above it was separated from the sample solution as ammonium molybdophosphate first of all and removed filtration through  $\text{IG}_4$  crucible. There is no effect of molybdate solution on the determination of the above-mentioned divalent cations.

The filtrate after the separation of phosphate was made upto a known volume. Convenient volume of the filtrate containing a mixture of calcium and lead (or  $\text{Cd}^{2+}$  or  $\text{Zn}^{2+}$ ) was titrated against standard 0.01 M EDTA solution using 4 drops Eriochrome Black T as indicator at a pH ~ 10 till the colour

TABLE I  
Complexometric determination of  $M^{2+}$ ,  $Ca^{2+}$

Sl. No.	$M^{2+}$ (mg)			Calcium (mg)		
	Theo.	Expt.	% Error	Theo.	Expt.	% Error
1	112.46	112.00	0.35	120.24	120.24	0.00
2	61.20	60.90	0.45	60.12	60.60	0.20
3	207.20	207.10	0.04	80.16	80.08	0.10
4	103.60	103.50	0.09	40.08	40.08	0.00
5	65.37	65.37	0.00	60.12	60.12	0.00
6	49.03	49.03	0.00	40.08	40.08	0.00
7	111.70	111.35	0.30	80.16	80.04	0.15
8	55.85	55.60	0.44	60.12	60.12	0.00
9	200.59	199.59	0.50	120.24	120.24	0.00
10	100.03	100.03	0.00	60.12	60.12	0.00

(Where  $M^{2+}$  for serial numbers 1 and 2 is Cd, 3 and 4 is Pb, 5 and 6 is Zn, 7 and 8 is Fe, 9 and 10 is Hg).

is changed from wine red to blue. The volume of EDTA solution consumed in the titration corresponds to the total content of  $Ca^{2+}$  and  $Pb^{2++}$  (or  $Cd^{2+}$  or  $Zn^{2+}$ ) in the filtrate taken. To the same volume of another aliquot of the filtrate 4 ml of buffer of pH 10 and 1.0 g of solid  $NH_4F$  were added and titrated against 0.01 M standard EDTA solution using Eriochrome Black T as indicator till the colour was changed from wine red to blue. The volume of EDTA consumed in this titration corresponds to the content of lead (or  $Cd^{2+}$  or  $Zn^{2+}$ ) alone. The difference between the titre values of the above experiments corresponds to the content of calcium in the filtrate taken. In the determination of a mixture of Calcium and Iron (or Mercury) to a convenient volume of the filtrate a known excess of standard 0.01 M EDTA was added and then back titrated with 0.01 M  $MgSO_4$  till the colour changed from blue to wine red. The volume of  $MgSO_4$  consumed in the titration corresponds to the total content of Calcium and Iron (or Mercury) in the filtrate taken. Calcium content in the same volume of another aliquot of the filtrate was masked as described earlier, and a known excess of 0.01 M EDTA was added and then back titrated with 0.01 M  $MgSO_4$  till wine red colour was obtained. From the volume of  $MgSO_4$  consumed in this titration the content of Iron (or Mercury) in the filtrate was calculated. The difference between the titre values of the above experiments

corresponds to the content of calcium in the filtrate. Representative sets of the results are given in Table I. The method suggested was found to be very rapid and accurate.

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#### SEPARATION OF IRON (III), COBALT (II) AND NICKEL (II) BY PAPER CHROMATOGRAPHY

IRON (III), cobalt (II) and nickel (II) have been separated by paper chromatography using several solvent mixtures by various workers<sup>1-7</sup>. We have studied the separation and determination of Fe (III), Co (II) and Ni (II) by ascending paper chromatography at various conditions using 85.0% acetone, 5.0% con. HCl and 10.0% methyl isobutyl ketone as solvent system. The minimum time required for the separation is 15 minutes, whereas the methods recommended so far require minimum of 90 minutes for the separation<sup>1-3</sup>.

All the chemicals used were of A.R. quality. One ml of each of the solution (1.0 M) of Fe (III), Co (II) and Ni (II) as sulphates was mixed together in a beaker and 5  $\mu$ l of this mixture was applied to the strips of Whatman paper No. 1 (30  $\times$  30 cm) at the base line and the paper strips were allowed to dry. The paper strips were then kept in the developing solvent, taken in a glass jar which was previously saturated with the vapours of the solvent and chromatogram was run for various intervals. The positions of these ions on strips were located with the aid of 0.1% alcoholic solution of rubeanic acid followed by exposing the strips to ammonia vapours. The  $R_f$  values of these ions have been calculated under various conditions.

After trying various solvents, it has been observed that reasonably good separation can be achieved within 15-30 minutes with a solvent mixture of 85.0% acetone, 5.0% con. HCl and 10.0% methyl isobutyl ketone although the best separation is possible within 45-90 minutes (Table I).

TABLE I

Effect of time on separation of Fe(III), Co(II) and Ni(II)

Solvent composition: 85 ml acetone + 5 ml HCl (32%) + 10 ml methyl isobutyl ketone

Time min.	R <sub>f</sub> Values			Separation
	Fe (III)	Co (II)	Ni (II)	
15	1.0	0.85	0.17	Good
30	1.0	0.81	0.15	Better
45	1.0	0.67	0.13	Best
60 to 90	1.0	0.64	0.08	do.

The presence of impurities like Zn (II) and Al (III) interferes with the separation of Fe (III), Co (II) and Ni (II), since they move with Fe (III). However, the presence of Cu (II) does not interfere. From the foregoing account it can be concluded that this method which involves methyl isobutyl ketone as one of the components of the solvent system renders quick and satisfactory separation of these three ions.

These three ions have been determined in micro quantities after their separation on paper strips using solvent extraction and colorimetric techniques. The experimental values in micro grams for Ni (II), Fe (III) and Co (II) were found to be 45.2, 18.4 and 44.4 against theoretical values, 48.7, 20.7, and 48.1 respectively.

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## A SIMPLE POTENTIOMETRIC METHOD FOR THE ESTIMATION OF BHC

GENERALLY, for the estimation of lower concentration of BHC (1, 2, 3, 4, 5, 6-Hexachlorocyclohexane, commonly called benzenehexachloride), a widely used insecticide, gas chromatographic<sup>1</sup> or colorimetric<sup>2</sup> methods are commonly employed. But most of the laboratories do not have the costly gas chromatograph equipped with either an electron capture detector or a microcoulometric detector. The other methods, usually employed for the estimation of this compound in its technical and commercial formulations<sup>2,3,4</sup>, are not very sensitive to low concentrations as that found in its aqueous solutions. As such, at present it is difficult to estimate traces of BHC in solutions. The potentiometric method described below is sensitive, simple and can be adopted even for residue analysis. The method is based on the hydrolysis of BHC under alkaline condition, to trichlorobenzene and inorganic chloride. The inorganic chloride thus released is estimated using a chloride ion-specific electrode. The concentration of chloride is taken as an index of the concentration of BHC. The procedure for the new method is given below.

Partition the BHC to hexane phase from a known volume of its aqueous solution, by repeatedly extracting its aqueous solution with 10 ml portions of *n*-hexane. Similarly, the hexane extracts of any other substance containing BHC can be used for analysis. The combined hexane extracts are transferred to a 50 ml test tube and the contents are evaporated to dryness on a water-bath at 60° C. Moisten the residue in the tube with 2 ml of distilled water, treat with 2 ml of 0.5 N ethanolic potash and keep the tube on a water-bath at 55°–60° C, till the contents are almost evaporated. Cool and extract the residue with 2 ml portions of water, finally making the extract to 10 ml. Mix well and determine the concentration of chlorides in the solution using a chloride ion-specific electrode (Beckman Silver-Silver chloride electrode or any other make similar electrode). The electrode potential is measured on a potentiometer (pH meter) having an expanded scale (Corning model No. 12 pH meter or its equivalent). Determine the concentration of BHC in the sample by referring to a calibration curve previously prepared by adopting the same procedure with 1 to 10 mg of BHC.

This method has been found quite satisfactory for solutions containing more than 0.1 ppm of BHC. Although the aqueous solubility of this compound is small, its concentration in water solutions can be estimated even in such small quantities, by partitioning the compound from a large volume of the solution to hexane phase, and then further con-

centrating the hexane solution. The same method can also be used for the estimation of residues of BHC in soils and plants, however, a suitable clean up procedure has to be adopted for such purposes before it is potentiometrically estimated to get rid of other chlorine containing compounds. The electrode potential should be measured on a sensitive instrument (preferably an expanded scale pH meter with mV markings), which can accurately show response to change of even one millivolt.

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#### HYDROCYANIC ACID CONTENT OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ) PEEL, AS AFFECTED BY FERTILIZER APPLICATION

CASSAVA (*Manihot esculenta* Crantz) a native plant of South America is being widely cultivated in the tropics. In Tamil Nadu and Kerala it is cultivated mainly for its tubers, a large quantity of which is utilised in industry for the manufacture of products like cassava flour, sago starch, etc. The peel from the tubers which is a waste product of the industry is used as a livestock feed. But one of the major deterrents to its use as a feed is the presence of poisonous hydrocyanic acid (HCN) in it. It contains a cyanogenic glucoside "linamarin" and an enzyme "linase". When the enzyme linase is brought into intimate contact with the linamarin as a result of mincing or injury to the tissues, the HCN is liberated which at higher dose causes instantaneous death to livestock by arresting cellular oxidation. Garner (1957) observed that an intake of plant material equivalent to HCN intake of 4 mg per kg body weight was lethal. Jennings (1970) reviewed and reported that cumulative or chronic poisoning by HCN from cassava has been associated with many illnesses including nervous diseases, goitre, etc. In this communication, the HCN content of the peel of a few promising types

of cassava and the effect of NPK fertilizers on it are reported.

Thirty promising cultivars of cassava were grown to maturity and pulled out from the soil. The peel was separated from the flesh and analysed immediately for its HCN content by adopting the colorimetric method of Indira and Sinha (1969). The results of analysis for HCN revealed that minimum amount of HCN was found to be in Kadayannallur and Noor rathal cassava varieties (125 mg/kg of fresh peel) and the highest amount of HCN was found in S-3 variety (1475 mg/kg). The mean value of HCN in the peel of different varieties of cassava was 599 mg/kg which is about fifteen times higher than its concentration of 41 mg/kg in the fresh edible portion of the tuber. There was large variation among the varieties for HCN content (CV 62%).

A significant positive relationship was found to exist between the HCN content of the edible portion of the tuber and of the peel ( $r = 0.781$ ). However, Sinha and Nair (1968) reported that there was no relationship between cyanogenic glucoside content in the rind and the flesh.

The effect of nitrogen, phosphorus and potash fertilizer application on the HCN content of peel of three varieties of cassava was also studied. It was observed that nitrogen application increased significantly the HCN content of the peel (756 ppm) at 150 kg.N/ha. This result is in consonance with that of Harms and Tucker (1973) who reported that the HCN content of Sudan grass was increased by increasing N application. Application of fertilizer P and K did not have any significant effect on the HCN content of cassava peel.

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# EFFECT OF ADRENALINE ON THE INDUCTION OF STREPTOZOTOCIN DIABETES

STREPTOZOTOCIN (SZN)<sup>5</sup> is an antibiotic derived from *Streptozotomycetes achromogenes*. Chemically, it is 1-methyl-1-nitrosourea derivative of D-glucose<sup>2</sup>. 2-deoxyglucose (2 DG) can protect beta cells against the diabetogenic effect of SZN<sup>2</sup>; 2 DG can readily enter cells and be phosphorylated<sup>4</sup>; the product of the phosphorylation diminishes glucose metabolism by inhibition of phosphoglucose isomerase<sup>6</sup> and possibly by reducing glucose uptake<sup>4</sup>. Since adrenaline (ADR) reduces the phosphorylation of 2 DG in skeletal muscle<sup>4</sup>, it is of interest to test whether ADR could alter the protective action of 2 DG against SZN diabetes.

The degree of diabetes was assessed by a comparison of the blood sugar curves obtained during glucose tolerance tests (GTT) carried out two days before and one week after treatment with SZN, in male albino rats (200 to 280 g body weight). The procedure for GTT was as follows: Fasting blood sample was taken from animals (fasted for 16 hours), anaesthetised with pentobarbitone (40 mg/Kg subcutaneous), a dose of 1.5 ml of 20% glucose solution per 100 g body weight was administered by stomach tube, and two (hourly) blood samples collected following glucose, from a wound on the tail tip and analysed for glucose<sup>1</sup>.

Preliminary experiments showed that: (1) 45 mg/Kg was a suitable dose of SZN for induction of marked diabetes; (2) simultaneous administration of ADR did not affect this action of SZN; and (3) 2DG, administered one week earlier, did not alter the GTT of otherwise normal rats. Three groups of rats received the following treatments, in addition to 45 mg/Kg of SZN administered intravenously in a fresh solution (pH 3.8 to 4.2, concentration 16.7 mg/ml).

Group A: ADR subcutaneously 25 min (100 micrograms) and 10 min (50 micrograms) before SZN.

Group B: 2DG, 1 g/Kg intravenous, in fresh solution (400 mg/ml) 15 min before SZN.

Group C: Both ADR and 2DG together as in above groups.

In Table I, the mean blood glucose values of each group, during GTT before and after treatment, are compared. The "area" under the GTT curve (equal to half of the sum of the blood glucose values at 0 and 120 min plus the value at 60 min, in mg hour per 100 ml) is a measure of the glucose tolerance of the animal<sup>3</sup>; the difference in GTT areas before and after treatment ( $\Delta$  area) in the same animal is an estimate of the degree of diabetes produced by the treatment.

It is seen that SZN produces marked diabetes even in the presence of ADR as indicated by the high  $\Delta$  area of Group A, in Table I, the values being comparable to those obtained with SZN alone in other experiments in this laboratory. The rats which received 2DG along with SZN (Group B) showed only a small, though significant,  $\Delta$  area indicating that 2DG affords almost complete protection against the diabetogenic effect of SZN. But, when ADR is given along with 2DG and SZN (Group C), there is a marked  $\Delta$  area similar to that in Group A. It thus appears that ADR can reduce, if not abolish, the protective effect of 2DG against SZN.

In view of the fact that ADR does not interfere with the diabetogenic effect of SZN, the demonstrated ability of ADR to annul the protective effect of 2DG is perhaps related to some interaction between 2DG and ADR. Since ADR can interfere with the phosphorylation of 2DG, as it appears in skeletal muscle<sup>4</sup>, it may be suggested that 2DG has to be phosphorylated, in order to exert its protective effect against SZN. However, it is difficult to envisage the role of 2DG-6-phosphate in

TABLE I

Group	No. of Rats	Treatment	Blood glucose mg/100 ml during GTT			Area (mg hr/100 ml)	$\Delta$ Area (mg hr/100 ml)
			Relation to Treatment	0 min	60 min	120 min	
A	4	Adrenaline + Streptozotocin	Before	95 $\pm$ 5	178 $\pm$ 27	89 $\pm$ 5	257 $\pm$ 40
			After	233 $\pm$ 67	469 $\pm$ 65	388 $\pm$ 101	779 $\pm$ 147
B	5	2-Deoxyglucose + Streptozotocin	Before	93 $\pm$ 3	143 $\pm$ 25	87 $\pm$ 4	248 $\pm$ 28
			After	113 $\pm$ 4	128 $\pm$ 35	119 $\pm$ 9	308 $\pm$ 21
C	4	Adrenaline + 2-Deoxyglucose + Streptozotocin	Before	100 $\pm$ 4	163 $\pm$ 21	100 $\pm$ 4	263 $\pm$ 23
			After	170 $\pm$ 24	385 $\pm$ 33	394 $\pm$ 77	653 $\pm$ 63

All values area: Mean  $\pm$  Standard Error of Mean. For details of treatment and GTT, see text. In paired  $t$  tests,  $\Delta$  area was significant ( $P < 0.05$ ) in all three groups.



antagonising the action of SZN, since it is not further metabolised in peripheral tissues<sup>6</sup>; the interference could be at the membrane level, with the uptake of SZN.

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#### LONG-TERM EFFECT OF NORGESTREL ON BIOCHEMICAL CHANGES IN RAT FALLOPIAN TUBE

NORGESTREL (13  $\beta$ -ethyl 17  $\alpha$ -ethinyl 19-nortestosterone; Wy 3707) has been reported to be one of the most potent contraceptive progestational steroids<sup>1</sup>. Little is known about the biochemical mechanism of its contraceptive efficacy. However, the biochemical changes in rat uterus under the influence of continuous low dose regimen of this steroid are well demonstrated<sup>2,3</sup>. But meagre attention seems to have been paid to the study of biochemical changes in Fallopian tube under such steroidal administration. The present study deals with the chemical composition of rat Fallopian tube after norgestrel administration under continuous low dose therapy.

Adult healthy albino female rats (Institute Colony) of regular estrus cycle were administered orally with dl-norgestrel dissolved in olive oil at the dose level of 0.3  $\mu$ g per rat per day without interruption. The dose of the steroid was calculated on the basis of the human antioviulatory dose<sup>4</sup>. A group of animals was sacrificed at each time intervals of 4, 8 and 12 months after drug feeding along with their respective control group. The animals fed with olive oil served as control. After the completion of scheduled feeding animals were sacrificed and Fallopian tube was carefully dissected out. The biochemical indices were determined in this organ. Protein and glycogen were estimated colorimetrically by the method of Lowry<sup>5</sup> and Montgomery<sup>5</sup> respectively. Lactic acid and acid soluble phosphorus were determined in protein free filtrate as described by Hawk<sup>6</sup>.

The protein content of the tissue recorded a decrease due to norgestrel treatment. The decrease was statistically highly significant ( $P < 0.01$ ) at 8 and 12 months treatment period. Glycogen level also registered a significant depletion ( $P < 0.05$ ) throughout the periods of treatment. Likewise, lactic acid content was significantly ( $P < 0.05$ ) diminished. But acid soluble phosphorus remained unaltered as compared to corresponding control values.

The long-term treatment with dl-norgestrel in continuous low dose regimen caused significant decrease in protein content. It has been reported in case of rat<sup>7</sup> and monkey<sup>8</sup> that the Fallopian tube as a component of the reproductive complex is quite sensitive to estrogen and progesterone. Norgestrel has been demonstrated by Edgren<sup>1</sup> to be one of the most potent antiestrogenic steroid. Since estrogen is known to have anabolic effect on its target organs such as uterus and Fallopian tube,

TABLE I  
Effect of norgestrel on biochemical changes in rat Fallopian tube (Mean values  $\pm$  S.E.)

Treatment		Protein (g/100 g)	Glycogen (mg/100 g)	Lactic acid (mg/g)	Acid Soluble Phosphorus (mg/g)
4 month	Control†	19.70 $\pm$ 0.70	158.8 $\pm$ 12.00	1.58 $\pm$ 0.020	0.635 $\pm$ 0.042
	treated	17.80 $\pm$ 0.20*	115.5 $\pm$ 2.00*	0.87 $\pm$ 0.21*	0.517 $\pm$ 1.02
8 month	Control	16.50 $\pm$ 0.64	162.5 $\pm$ 2.50	1.18 $\pm$ 0.075	0.590 $\pm$ 0.024
	treated	12.55 $\pm$ 0.41**	135.0 $\pm$ 10.00*	0.16 $\pm$ 0.090**	0.498 $\pm$ 0.034
12 month	Control	22.43 $\pm$ 0.52	160.8 $\pm$ 7.00	2.08 $\pm$ 0.032	0.340 $\pm$ 0.041
	treated	17.50 $\pm$ 0.65**	130.2 $\pm$ 8.40*	1.13 $\pm$ 0.038**	0.380 $\pm$ 0.038

† Animals served with 1 olive oil only. \* Significant at 5% ( $P < 0.05$ ). \*\* Significant at 1% ( $P < 0.01$ ).

it appears that the decrease in the protein content as a result of norgestrel administration might be due to its antiestrogenic profile.

Glycogen, a carbohydrate reserve, was also found to be diminished significantly in treated group. This lowering of the level of glycogen may be due to decreased rate of synthesis because of antiestrogenic action of the steroid as glycogen is an estrogen dependent substrate<sup>8</sup>. However the breakdown of glycogen does not appear to be through EMP pathway since the level of lactic acid was also lowered significantly. Similarly, no evidence is available in this study for anabolic activity, where lactic acid was being rapidly utilized through Krebs cycle at a rate higher than the normal. This contention is further supported by the values of acid soluble phosphorus which showed no change after treatment with this drug. Since the production of energy means trapping of Pi for the formation of ATP.

To sum up the position, it may be stated that long-term treatment with norgestrel in continuous low doses induced a general lowering of the metabolic status of the Fallopian tube, which may be one of the reasons of its contraceptive efficacy along with the other major mechanism of action like general retardation of metabolic activity of uterus and ovary<sup>9-10</sup>, etc.

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# EFFECT OF ARHAR MOSAIC VIRUS ON NODULATION, NITROGEN VALUE AND NITROGEN FIXATION BY SANNHEMP (*CROTALARIA JUNCEA* L.)

SANNHEMP is an important leguminous crop which is mainly cultivated for green manure, fodder and for fixing atmospheric nitrogen to enrich the soil. Several plant viruses are reported to infect this crop in nature but their effect on its nitrogen value nodulation and nitrogen fixation has not been studied. The present communication reports our observations on arhar mosaic virus strains (ASM and AMM) infection on the above aspects of Sannhemp (*Crotalaria juncea* L.).

Arhar mosaic virus strains (ASM and AMM) were originally isolated from field grown arhar [*Cajanus cajan* (L.) Millsp.] and their culture maintained in arhar cv. S-8 in glass house. Both the strains infect *Crotalaria juncea* L. and produce mosaic mottling with reduced growth but ASM is more severe than AMM in its reaction. Strain ASM has a DEP 1 : 1000,000, TIP 80° C and longevity *in vitro* 11 days while the other strain AMM has a DEP 1 : 1000,000, TIP 60° C and longevity *in vitro* 16 days at room temperature (Min. 48–62, Max. 48–100° F). Both the isolates are infectious only to leguminous host. The inoculum of both the strains was prepared separately by macerating the young infected leaves in a mortar and squeezing the pulp through muslin cloth. The infective sap was diluted 1 : 10 with distilled water before use. Clay pots of 20 cm diameter containing 5 kg of sterilized mixture of sand, loam and compost (1 : 1 : 2) were taken. Sixty such pots having five seedlings of Sannhemp in each were divided into three sets of 20 pots. Seven-day old seedlings of first and second sets were inoculated separately with ASM and AMM strains, respectively, while the third set was left as healthy control. The plants were harvested after 45 days of inoculation. At the time of harvest data on growth, nodulation, nitrogen value of plant and nitrogen percentage of soil were recorded. The growth data was recorded as described by Singh and Bhargava<sup>1</sup> and nodulation by the methods given by Tu *et al.*<sup>2</sup>. Total nitrogen of composite dried plant or soil material was estimated by a colorimetric procedure described by Snell and Snell<sup>3</sup> and Misra<sup>4</sup>, respectively, by using hilger pattern Biochemical Absorptiometer with filter No. 43. The nitrogen value of plants is the total sum of the nitrogen present in different parts of the plant and the nitrogen added or fixed to the soil which is obtained from the difference between the nitrogen present in the soil at the time of harvest and nitrogen of pot soil at the time of seeding.

TABLE I

*Effect of arhar mosaic virus on growth, nodulation and nitrogen content\* of Sannhemp*

	Treatment				
	Healthy	ASM	**	AMM	**
Height of shoot (cm)	74.9	54.1	-27.8	55.6	-25.8
Length of root (cm)	26.5	24.7	-6.8	26.0	-1.9
Fresh wt. of shoot (g)	12.83	9.49	-26.6	-10.23	-20.3
Fresh wt. of root (g)	2.47	1.21	-51.0	1.31	-47.0
Dry wt. of shoot (g)	2.72	1.96	-27.9	1.65	-39.3
Dry wt. of root (g)	0.38	0.43	+13.2	0.38	00.0
No. of nodules/plant	24.0	4.0	-83.3	10.0	-58.3
Fresh wt./ nodule ( $\text{g} \times 10^{-2}$ )	0.200	0.260	+30.0	6.255	+27.5
Dry wt./ nodule ( $\text{g} \times 10^{-2}$ )	0.035	0.030	-14.3	0.030	-14.3
Volume/nodule ( $\text{ml} \times 10^{-2}$ )	0.250	0.280	+12.0	0.270	+8.0
% Total nitrogen in shoot	2.17	2.50	+15.2	2.35	+8.3
% Total nitrogen in root	1.90	1.98	+4.2	2.22	+16.8
% Total nitrogen in nodule	2.76	3.00	+8.7	2.98	+8.0
Nitrogen value (g)	0.08942	0.06060	-32.2	0.056151	-37.2
Total % soil nitrogen	0.68	0.60	-11.8	0.63	-7.4
Total nitrogen added to soil	0.46	0.38	-17.4	0.41	-10.9

\* % nitrogen/100 mg dry wt.

\*\* % increase (+) or decrease (-) over healthy plant.

Results presented in the table indicate that both the virus strains reduced the shoot height, root length, fresh and dry weight of shoot. Although the diseased root of sannhemp showed a reduction in fresh wt. but its dry wt. was higher in comparison to healthy ones. Both the strains reduced the nodule number per plant and their dry weight but increased the nodule size and fresh weight. Arhar mosaic virus increased total nitrogen content in shoot and root than their healthy counterparts, but the total nitrogen value per plant was higher in healthy than infected ones. Before seeding the pot soil contained 0.22% nitrogen but at the time of harvest percentage of soil nitrogen was increased. Maximum increase was noted with healthy sannhemp plants followed by AMM and ASM infected plants.

Arhar mosaic virus infection reduced the growth and fresh weight of sannhemp plants but increased the dry weight of the root in comparison to healthy plants. Stunting of growth is the most common

symptom produced by the virus infection<sup>5</sup>. The virus infection decreased the nodule number and their dry weight but increased their size and fresh weight. A reduction in nodule number was also reported in white clover plants infected with clover phyllody virus<sup>6,7</sup>, and soybean infected with soybean mosaic virus (SMV) and pod mottle virus (BPMV)<sup>2</sup>. The reduction in number of nodules was concomitantly associated with an increase in their fresh weight and size in virus infected plants. Tu *et al.*<sup>2</sup> suggested that reduced nodulation in infected plants was probably caused by viral multiplication leading to physiological changes of reduced photosynthesis, increased respiration and the imbalance of auxins and enzyme levels. The higher dry weight in root and lower in nodule of diseased plants can be explained as suggested by Gibson<sup>8</sup> that it might be due to complex auxin relationship or competition in roots and nodules for carbohydrates. The increase or decrease in dry weight seems to be related to carbohydrate accumu-

lations. A higher percentage of nitrogen was found in the nodules of infected plants. Similar findings were also reported by Tu *et al.*<sup>11</sup> and Rajagopalan and Raju<sup>9</sup> in SMV infected soybean and in *Dolichos* enation mosaic virus infected *Dolichos lab lab*, respectively. It is believed that this higher nitrogen percentage in the nodules is due to insufficient utilization of nitrogen by infected plants. Arhar mosaic virus reduced the total nitrogen value of plants. According to Orlob and Arny<sup>10</sup> this decrease is due to the inhibition of protein synthesis or increased rate of degradation of proteins in infected plants.

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#### EFFECT OF SOME MYCOTOXINS ON THE INFECTIVITY OF TOBACCO MOSAIC AND TOBACCO RING SPOT VIRUSES

ALTHOUGH plant virus inhibitors have been found in a number of species of higher plants and fungi, relatively few have been characterized chemically<sup>1,2,10</sup>. In the earlier studies Rao and Raychaudhuri<sup>6</sup>, Sharma and Raychaudhuri<sup>8</sup>, used the crude culture filtrates of *Trichothecium roseum* and *Aspergillus niger* respectively and reported that they are inhibitory to Potato virus X. Recently Kang *et al.*<sup>4</sup> reported that the culture filtrate of *Aspergillus flavus*, containing aflatoxins, inhibited the infectivity of vegetable marrow mosaic virus upto 70 to 80%.

Subbarayudu *et al.*<sup>9</sup> reported the effect of aflatoxins on cowpea mosaic virus infectivity.

With the end in view, we have isolated aflatoxins from the culture filtrates of *Aspergillus flavus* and separated by the method of Pons *et al.*<sup>5</sup>. Citrinin was extracted from the culture filtrate of *Penicillium citrinum*<sup>7</sup>. The T-2 toxin was obtained as a gift from Dr. O. Shottwell. The toxin solutions were prepared by dissolving a known amount of toxin in chloroform, to which later distilled water was added. After evaporating the chloroform, 500 and 1000 ppm toxin solution were prepared.

In the present studies, *Nicotiana tabacum* var. *xanthi-ne* was used as a local lesion host for tobacco mosaic virus (Johnson's No. 1 strain) and *Vigna sinensis* Savi for tobacco ring spot virus (Brinjal isolate). For testing the effect of these toxins, the standard inocula for these two viruses were prepared by communizing young infected leaves. The sap was filtered through double layered muslin cloth and diluted with distilled water to have 1:5 dilution which gave countable discrete local lesions on the above hosts. Different concentrations of each toxin were mixed with equal quantity of the virus inocula and incubated for 1 hr at room temperature (32°C). For control treatment, distilled water, from which chloroform was evaporated, was added to the virus inoculum, instead of toxin solution. After one hour incubation the inoculations were made by using half leaf method with inoculum wet cotton wad on the leaves previously dusted with celite. All the experiments were replicated three times and two independent experiments were conducted to confirm the results. The data obtained in both the experiments were averaged and presented in Table I.

It is quite obvious from the data given in the table that aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>), citrinin and T-2 toxin have inhibitory effect on tobacco mosaic virus and on tobacco ring spot virus. Aflatoxins, citrinin and T-2 toxin both at 500 and 1000 ppm inhibited the local lesion production of both the viruses and the percentage of inhibition ranged between 62.5 to 100% for TMV and 59.7 to 98.7% for TRSV respectively. In 1972, Kang *et al.*<sup>4</sup> reported 70 to 80% inhibition of vegetable marrow mosaic virus, with the culture filtrate of *Aspergillus flavus* containing aflatoxins. Citrinin also markedly inhibited local lesion formation on *Nicotiana glutinosa* leaves infected with TMV<sup>11</sup>. Earlier Rao and Raychaudhuri<sup>6</sup>, Sharma and Raychaudhuri<sup>8</sup> used only the crude culture filtrate of fungus without isolating the actual inhibitory substance. But in the present studies the toxins were isolated and the studies reveal that the inhibitory substance present in the crude culture filtrate of these fungi may be aflatoxins in the case

TABLE I

Effect of mycotoxins on the inhibition of local lesions

Treatment	Con- cen- tration of myco- toxin	% inhibition of	
		TMV	TRSV
Control	a	0(96)	0(77)
Aflatoxin B <sub>1</sub>	a	62.5	59.7
	b	77.0	68.8
B <sub>2</sub>	a	83.3	84.4
	b	87.5	87.0
G <sub>1</sub>	a	70.8	72.7
	b	81.2	80.5
G <sub>2</sub>	a	80.2	76.6
	b	85.4	81.8
Citrinin	a	75.0	74.0
	b	83.3	84.4
T-2 Toxin	a	97.9	97.4
	b	100.0	98.7

a: represents 500 ppm and b: 1000 ppm.

Figures in parenthesis are actual number of local lesions.

of *Aspergillus flavus* and citrinin in *Penicillium citrinum*, which greatly decreased the infectivity of the viruses under study.

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#### TUBER ROT OF *CYCAS BEDDOMI* DYER. CAUSED BY *FUSARIUM EUISEI* (CORDA) SACC.

In the rainy season (August to November) of 1971, a tuber rot of *Cycas beddomi* Dyer., an ornamental plant with feathery and evergreen leaves, was observed in Shri Venkateswara University botanical garden. It is an endemic plant occurring only in Seshachalam Hills (1000-3000' msl), Andhra Pradesh, India (Gamble and Fischer, 1956). Its occurrence in any other part of the world has not been so far reported. In the subsequent year also a high mortality (76%) of the plants was observed.

The initial manifestation of the disease is yellowing of the leaves in an ascending order. In the early stages of the disease development, the freshly sprouting foliage showed small scale-like brown leaflets (Fig. 1 C) and in later stages almost devoid of leaflets. As the disease progressed, the growth of the terminal bud is checked. When such plants were pulled up, they were found to be almost devoid of coralloid roots. The infected roots become brownish with almost discoloured vascular roots become brownish with almost discoloured vascular bundles in the initial stages and subsequently the entire ground tissue degenerated leaving tracheids encircled by a thick tubular sheath (Fig. 1 A). The rotting progressed from the lower side of the tuber towards terminal bud. In severe cases the entire ground tissue of the tuber including the terminal bud became rotten leaving fibres and tracheids (Fig. 1 B).

Almost all isolations from infected roots and tuber yielded the fungus, *Fusarium*. Isolations from rhizosphere soil carried out according to the method of Warcup (1950) gave *Fusarium* as the principal fungus. The fungus was purified by single spore isolation.

The organism grows luxuriantly on Potato dextrose agar (PDA) and Czapek-Dox agar; cottony with light brown to dull-pink substratum; both sterile and fertile hyphae are irregularly septate and 3-6 µ thick. Chlamydospores are mainly intercalary;

round; 6–15  $\mu$  in diameter. Both micro and macroconidia are present. Conidiophores are branched and sparingly scattered. Macroconidia are mostly 3–5 septate; pale brown; typical of a twisted spindle with parabolic curvature; measuring 12–45  $\times$  2.5–5.6  $\mu$  (3 septate) and 25–75  $\times$  2.5–5.7  $\mu$  (5 septate). Microconidia are numerous, non-septate, oval and 8.2–14.1  $\times$  3.1–4.2  $\mu$  in size. The fungus was identified as *Fusarium equiseti* (Corda) Sacc.

The fungus is able to grow under a wide range of temperatures. There is no growth at 5° and 44° C. The optimum temperature for growth is 25° C.

The fungus was grown in 3% oatmeal-sand for ten days and inoculated to sterilized soil at 5% inoculum level (W/W). The inoculated soil was taken in earthenware pots (12" size). Two to three years old plants showing no damage of the tuber and roots were planted in the pots. A set of 20 plants were maintained in fern-house, (23°  $\pm$  5° C) and another set in direct sunlight (30°–41° C). The control plants grown in sterilized soil and another two sets of plants raised in pots containing "sick soil" (the soil in which the disease was previously recorded) were maintained under the aforesaid conditions. Initial symptoms, i.e., the yellowing of the leaves were observed after 15 days. The rotting of roots and tuber was much severe in plants kept in low temperatures (fern-house). A 100% disease was noticed in the fungus-inoculated soils and 80% rotting was recorded in plants raised in "sick soil". In the plants kept in high temperatures (in direct sunlight), 60% of the disease in inoculated soil and 20% of the disease in "sick soil" was noticed. No disease was observed in control plants. Reisciations from the diseased plants (grown in inoculated pots) yielded a fungus similar to the parent culture.

Hitherto, no disease was recorded on this host. Hence the present report appears to be a new record.

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## SEGREGATION OF NON-LYSOGENIC CELLS FROM LYSOGENIC CLONES OF *SALMONELLA* *TYPHIMURIUM*

It has been reported<sup>1</sup> that temperate phage 547 of *Salmonella typhimurium*, Strain 547 transduced histidine, leucine, and tryptophan markers into recipient cells (H L T) of *Salmonella typhimurium*, Strain 533 and that only a portion of transduced cells (clones) were lysogenised suggesting that transduction and lysogenisation were independent processes in this system studied. It was of further interest to know whether or not all cells of the lysogenic clone were lysogenized.

The following procedure was followed:

The lysogenised clones (which were tested for the ability of releasing of phage 547) were grown overnight in tubes containing brain-heart infusion broth medium. The cells were spun down by centrifugation and resuspended in saline. Appropriate dilution of each suspension was treated with antiphage 547 serum to inactivate the free phage and 1 ml of this diluted culture was plated on minimal medium containing required amino acids<sup>1</sup>. After two days of incubation colonies have appeared on the plates and the test for lysogenisation was made by gently pouring a top agar layer containing sensitive *Salmonella typhimurium* (TC) cells<sup>1</sup>. If phage was released from any colony an area of lysis appeared around it suggesting that the clone derived out of a single cell was lysogenic. The number of clones showing such clear areas was recorded after one day of incubation. The results of such tests are shown in Table I.

TABLE I  
Showing percentage of lysogenic and non-lysogenic cells due to segregation

Transduced clone	No. of clones released phage	No. of stable lyso-genic cells	% Lyso-genic cells	% Non-lysogenic cells
Hist <sup>+</sup>	440	40	9.1	90.9
Try <sup>+</sup>	200	22	11.0	89.0
Leu <sup>+</sup>	420	19	4.5	95.5

These results indicated that not all cells within a lysogenised clone (originally isolated) were lysogenic but only a fraction varying from 4.5% to 11.0%. This finding gives some light on the establishment of lysogenisation. A similar phenomenon was observed by Luria *et al.*<sup>2</sup>, who found segregation of sensitive non-lysogenic and immune-lysogenic cells among the descendants of lysogenised cells derived from infection with temperate phage p 22. This suggested that lysogenisation was not

established immediately after infection with temperate phage but after one or more divisions of the original infected cell. The results obtained using phage 547 of *Salmonella* also support the above view.

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#### BREEDING OF THE COMMON HOUSE FLY, *MUSCA DOMESTICA* L. FOR PHYSIOLOGICAL STUDIES

GRADY<sup>1</sup>, Hockenyos<sup>2</sup>, Richardson<sup>3</sup>, Roy and Siddons<sup>4</sup>, David and Harvey<sup>5</sup>, and Basden<sup>6</sup>, developed different media for rearing larvae and adults of *Musca domestica* L. Deoras<sup>7</sup> prepared a sophisticated diet for rearing flies throughout the year under natural lighting conditions. This diet consists of many ingredients including banana, milk, cane-sugar, agar-agar and yeast. Thus its preparation requires many constituents which may not be readily available everywhere. To overcome this handicap, a simple rearing medium was tried.

The flies were collected from the field and kept in a cage containing sugar crystals and moist filter-paper, serving as food and water sources. From this a copulating pair was captured in a test tube and released in a separate cage. This pair served as the source for further multiplication. In the cage were placed a Petri dish containing milk-soaked cotton, a water-filled staining jar inverted in a Petri dish lined with filter-paper, and a watchglass containing sugar crystals. The milk and water dishes were changed daily. The flies fed on milk and sugar, and laid eggs in bunches in the milk-soaked cotton. The dishes containing eggs were transferred to a separate breeding cage and placed in a trough containing moist sawdust. The dishes, in which eggs were laid on the same day, were placed in the same breeding cage so that the flies of a known age could be obtained. The eggs hatched within 6 to 12 hours and the larvae buried themselves in the milk-soaked cotton. Fresh milk was added every day to keep the cotton just moist because an insufficient quantity of milk or too much milk in the cotton caused premature larval migration. Larvae took 4 to 6 days to grow

and then migrated into the sawdust. Addition of milk was then stopped. The larvae pupated in the sawdust. Some, however, migrated into the sawdust before maturity and this led to poor development.

The pupae were transferred to a jar covered with a piece of thin cloth. Flies emerged 4 to 6 days after pupation and the flies of the same age were stocked together in cages and fed on milk. These copulated in 4 to 8 days after emergence and laid eggs 4 to 6 days later, and the cycle was repeated.

The rearing was done under normal laboratory temperature and humidity conditions which ranged from 24° to 41° C and 20% ± 15% RH in summer and from 6° to 24° C and 25% ± 15% RH in winter.

Egg-laying was delayed in winter as the developing stages needed warmer conditions. Hence the Petri dishes containing eggs and larvae and the jars containing pupae were placed in an incubator at 37° C. A Petri dish filled with water was also placed in the incubator to maintain proper humidity.

The progeny from a single pair of flies had been maintained for five years under the aforesaid conditions and therefore milk appears to be a satisfactory diet for the larvae and adults both. Development proceeded normally even when Glaxo baby food was substituted for fresh milk.

Occasional fungal contamination of the breeding cages was avoided by rinsing them with 0.01% solution of mercuric chloride once a week. Sometimes during winter, fungus attacked the adult flies severely. The affected flies can be distinguished by their swollen abdomen and minute hyphae projecting out through the intersegmental membrane in the abdomen. The flies kept in sunlight had severe attack as compared to those indoors.

It may be concluded that the diet described here for rearing house flies is more handy than those described so far.

I wish to express my deep gratitude to Dr. R. Rakshpal for constructive criticism.

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ON A NEW RECORD OF *ANOPLOPHRYA*  
*LUMBRICI* (SCHRANK) (PROTOZOA: CILIATA)  
FROM A NEW HOST, *PHERETIMA PEGUYANA*  
(ROSA) FROM CALCUTTA

DURING our study of the parasitic protozoa of earthworms, we came across a number of ciliates from the seminal vesicles of a few specimens of *Pheretima peguyana* (Rosa); the latter were collected from the compound of St. Cathedral Church, Calcutta.

The ciliates were studied alive before being fixed in Schaudinn's fluid, stained in Heidenhain's iron-haematoxylin and counter-stained in eosin for further detailed studies.

**Description.**—Body elongate-oval with sub-truncate posterior end; dorsal side convex, ventral side concave. Ciliary striae close; 3 contractile vacuoles observed in living specimens. Macro-nucleus more or less ribbon-shaped, extending almost the entire length of the body. Micronucleus spherical, situated by the side of macronucleus.

**Dimensions**—Length: 180–250  $\mu$ ; Width: 50–70  $\mu$ ; Macronucleus: 100–120  $\mu$ .



FIG. 1. *Anoplophrya lumbrici* (Schränk),  $\times 450$ .

**Remarks.**—This species was originally described by Schränk (1803). Heidenreich (1935) redescribed it and considered *A. lloydi* Ghosh a synonym of *A. lumbrici*. Bhatia (1936), however, re-established *A. lloydi* as a valid species. Since the specimens described here resemble those described as *A. lumbrici* by Heidenreich, these are being identified as *A. lumbrici*. This is the first record of the occurrence of *A. lumbrici* from a new host, *Pheretima peguyana*, in India.

The authors are grateful to Dr. S. Khera, Dr. T. G. Vazirani and Shri K. N. Nair of the Zoological Survey of India for constant encouragement and help in various ways.

Zoological Survey of India. M. K. BISWAS.  
Calcutta-12, February 13, 1974. R. N. MOOKHERJEE.

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STOMATOGENESIS IN THE LEAVES OF  
*CLERODENDRUM PHLOMIDIS* L. f.

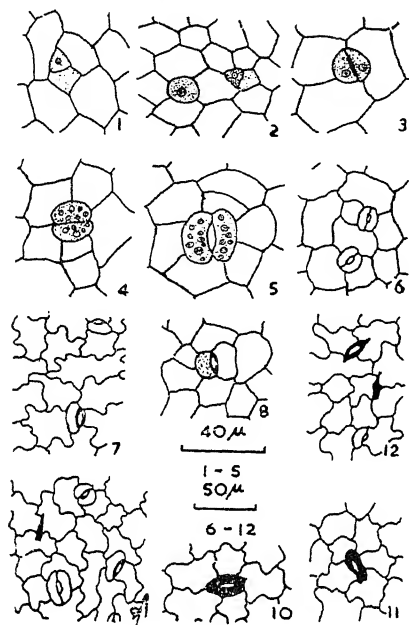
METCALFE AND CHALK<sup>1</sup> have reported that the stomata in *Clerodendrum* are of ranunculaceous type (not accompanied by definite subsidiary cells). Krishnamurthy and his co-workers<sup>2</sup> also observed the same type of stomata in the leaves of *C. phlomidis*. Recently Fryns-Claessens and Van Cotthem<sup>3</sup> stressed the importance of the ontogenetical studies of the stomata, especially of the anomocytic ones, as this type can be formed in two ontogenetic ways (perigenous and mesoperigenous). There is no report on the stomatal ontogeny in the entire genus though Inamdar<sup>4</sup> studied the development of the stomata in some other taxa of this family (Verbenaceae). Hence the present note deals with the ontogeny and structure of the stomata in the leaves of *C. phlomidis*. The terms, used here, are taken from Fryns-Claessens and Van Cotthem<sup>3</sup>.

The development of the stoma begins with the transverse division of the protodermal cell, producing two unequal cells. The smaller cell functions as the meristemoid and the larger one becomes indistinguishable from the other cells after some time.

The meristemoids are usually triangular or trapezoidal, surrounded by a minimum of 3–4 protodermal cells (Figs. 1–3). They are well distinguished from the other cells by their denser cytoplasm and larger nucleus. This meristemoid enlarges and becomes almost spherical (Fig. 2) before it divides vertically to form two equal guard cells (Fig. 3). At this stage the guard cells are surrounded by 3–5 neighbouring cells of comparatively smaller size (Fig. 4). The difference in the size between the guard cells and the neighbouring cells is attributed to their independent origin and rapid development of the meristemoid than the latter. As the stoma matures, the neighbouring cells increase in their number by supplementary radial divisions (Figs. 4, 5). Thus the mature stomata are accompanied by 5 to 6 undifferentiated neighbouring cells of various shapes (Figs. 6, 7). Simultaneously their walls become slightly sinuous on the upper and deeply sinuous on the lower epidermis. Sometimes the neighbouring cells divide



tangentially with the result one or two of them may look smaller (Figs. 4, 5).



FIGS. 1-12. *Clerodendrum phlomidis*. Figs. 1-5. Ontogeny of stomata. Fig. 1. A meristemoid cutting off from the protodermal cell. Fig. 2. Enlarged and spherical meristemoid. Fig. 3. Vertical division of the meristemoid producing two guard cells. Figs. 4, 5. Perigenous neighbouring cells of the stomata showing radial and tangential divisions. Fig. 6. Mature stomata of upper epidermis. Fig. 7. Abnormal stoma of lower epidermis with two epidermal cells replacing a guard cell. Figs. 8, 9. Stomata flanked by a single guard cell and a large cell. Figs. 9-12. Lower epidermis showing abnormal stomata with thick-walled abortive pores.

Evidently the meristemoids directly act as guard cell mother cells and the neighbouring cells are derived from the other protodermal cells. Hence these stomata are regarded as aperiogenous type following the recent classification proposed by Fryns-Claessens and Van Cotthem<sup>3</sup>. The present study also supports the view of the previous workers<sup>3-5</sup> that the meristemoid is the smaller meristematic daughter cell formed as a result of the division of the protodermal cell.

Though the leaves are amphistomatic, only the lower epidermis show the abnormal stomata as frequently as the normal ones. Rarely do they also occur on the upper surface. Similar stomatal anomalies were observed on the lower epidermis of several members of Solanaceae<sup>6</sup> and *Nelumbo nucifera*<sup>5</sup>. The stomatal abnormalities include the stomata with a single guard cell and the degenerated stomata. In the first type, either one of the guard

cells is transformed into a large thin-walled cell (Figs. 8, 9) or the two neighbouring cells crush one of the guard cells completely. In the latter case the stomatal pore is flanked by a single guard cell on one side and two ordinary epidermal cells on the other (Fig. 7). The second type is caused by the obliteration of the two guard cells by the encircling cells (Figs. 10, 11). They, however, do not disappear altogether as reported in the leaves of *Nelumbo nucifera*<sup>5</sup>. On the contrary, these abnormal stomata are represented by close or open, abortive thick-walled pores encircled by 3-5 neighbouring cells (Figs. 9-12) as what has been observed in some members of Solanaceae<sup>6</sup> and Rubiaceae<sup>7</sup>. Previous workers<sup>2</sup> could not find the abnormal stomata in this species. This may be due to the fact that the materials, they studied, were young. In the present study also, no abnormal stomata could be seen in the young and developing leaves. This observation confirms the contention of Ahmad<sup>6</sup> that these anomalous stomata are formed as a result of the degeneration of mature stomata rather than due to developmental disturbances.

I am highly indebted to Professor S. L. Basu and Dr. K. H. Krishnamurthy for their kind encouragement.

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#### INHERITANCE STUDIES ON CERTAIN PEA MUTANTS \*

KALLOO<sup>1</sup> isolated several chlorophyll mutants induced through gamma irradiation of dry pea seeds. This communication deals with a study of the manner of inheritance of four of the above chlorophyll mutants, viz., light yellow, light green, white spotted leaves and orange-yellow spotted leaves. A spontaneous mutant, yellow pollen, was also studied.

TABLE I  
Segregation of certain mutants in  $F_2$

Culture number	Name of mutant	F <sub>2</sub> Segregation No. of plants		Expected ratio	$\chi^2$ value 1 d.f.	P Value
		Normal	Mutant			
61	Light yellow	105	30	3:1	0.556	>0.30
62	Light green	61	19	3:1	0.067	0.80
63	White spotted leaves	219	15	15:1	0.026	>0.80
64	Orange-yellow spotted leaves	73	21	3:1	0.355	>0.50
65	Yellow pollen	56	19	3:1	0.004	0.95

Each mutant was crossed reciprocally with a normal plant during 1969-70. In 1970-71, the seeds of parents and  $F_1$  including reciprocals were grown in the field.  $F_1$  plants were selfed to get seeds for raising  $F_2$  population next year.  $F_2$  plants were classified into different phenotypes.  $F_1$  plants were all normal; reciprocal crosses showed absence of any maternal effect. The  $F_2$  data presented in Table I showed that all the mutants were recessive and showed a monogenic segregation of 3:1 except the white spotted leaves which showed a duplicate ratio of 15:1.

Blixt<sup>2</sup> showed that the chlorophyll mutants occurred not only in different forms but their expression also varied in different degrees due to multiple allelism. Light yellow and light green mutants could be grouped under *flavo-viridis* while the other could be grouped as *vario-maculata*—also a recessive mutant<sup>2</sup>.

Spontaneous occurrence of yellow pollen in pea was earlier reported by Murfet<sup>3</sup>. A similar mutant was observed in the present study also. Murfet<sup>3</sup> designated the allele for yellow pollen by *yp*.

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#### REPLICATION OF SURFACE FEATURES OF SAND GRAINS OF SIZES 0.5 TO 4.0 MM FOR ELECTRON MICROSCOPY

KNOWLEDGE of size, shape and external features of minerals, rocks and sands is sometimes needed for determining the crystal structure, nature of origin, growth, and also the reaction of minerals to physical and chemical forces imposed by man or nature<sup>1,2,4</sup>.

There is no universally correct method of producing surface replicas for electron microscopy since different specimens require variations of one or two basic techniques to be developed for it. Carbon is the most popular replicating material but simultaneous rotation of specimen during carbon evaporation has been known to yield tougher films. Moreover carbon films are unaffected by strong acids and almost all solvents.

In an attempt to replicate the surface features of sand grains of sizes 0.5-4 mm disaggregated from the friable sandstones from the tertiary sediments around Kotdwara Garhwal, Himalaya, various usually followed methods<sup>1-3</sup> were tried. Unusual large size with sharp contour elevations of the sand grains would rupture the replica film and no worthwhile features could be replicated. A slightly modified attempt was therefore made as follows.

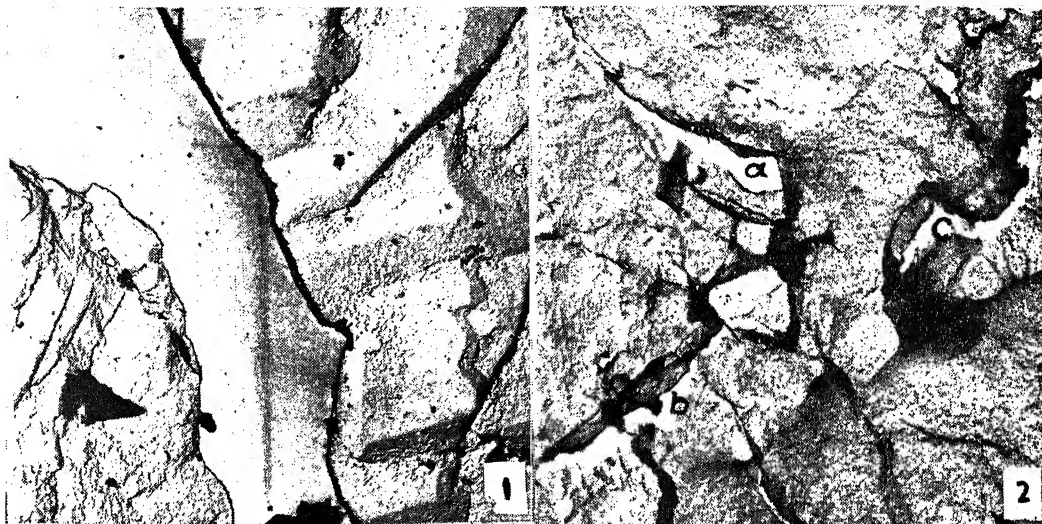
Cellulose acetate replicating tape was softened in acetone and mounted on a clean glass slide, cut to 2 cm squares earlier. Sand grains were then placed on this soft tape and pressed gently with another glass slide to obtain their surface impressions on the tape. On drying, the sand grains could be easily removed with a slight pressure by a needle or can just be picked up with a pointed

\* Part of a Ph.D. Thesis submitted to the Banaras Hindu University, Varanasi, in 1973 by the second author.

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forceps leaving their surface impressions on the tape. This slide was shadowed with Gold/Palladium (60/40) at 30° angle. The shadowed slide was then glued to the rotating grid holder inclined at an angle of 10–20° to the vertical and facing down the evaporating carbon electrodes about 8 centimeters below. The electrodes were carefully shaped and arranged to get the specimen slide within the cone of evaporation upwards. Carbon was evaporated with simultaneous rotation of the specimen at a speed 100 rpm, keeping the chamber pressure below  $10^{-4}$  torr in Edwards EI2E vacuum

ing concentrations of 30%, 50%, 90%, 100% acetone failed, as milkiness would appear and the replicas on observation in the Electron Microscope were found to be superimposed with a mosaic background. No such structure was seen to occur when the replicas were carefully fished out, on unsupported copper grids, directly from the acetone. Figures 1, 2 indicate the results obtained with this method, which involved no etching of sand grains with Hydrofluoric acid. Reproducibility of results with the above method has been found to be very high.



FIGS. 1–2. Fig. 1. Contour elevations and depressions well replicated, Mag.,  $\times 7,750$ . Fig. 2. Highly irregular weathered surface of sand grain showing tendency to rupture at sharp edges (a, b, c). Mag.,  $\times 15,000$ .

coating unit. The replica was then backed with a Formvar film by dipping the slide for 20 seconds, and vertically removing with care from a 0.25% Formvar solution in chloroform. After drying for 20 minutes, 4 to 5 mm squares were cut from the tape on the glass slide, with a very sharp razor blade. These squares which are easily separated from the slide are transferred to a petri-dish full of acetone, with a pointed forceps. Generally these squares dip into acetone but care should be taken to see that the tape side remains downwards while dipping. Replicas have been found to rupture into pieces when the tape is upwards during its dissolution in acetone. The replicating tape is dissolved in acetone for over 12 hours followed by two changes of acetone for 20 minutes each for removing any debris sticking to the carbon replica backed with formvar, that is left behind. Our attempt to slowly dissolve the replicating tape by transferring the replica squares in gradually increas-

Specimens thus prepared were observed on Philips EM-300 Electron Microscope with condenser lens  $C_1$  off.

Grateful acknowledgements to Prof. A. K. Verma, Department of Geology, University of Delhi, for specimen sand grains.

Nuclear Research Lab.,

Indian Agricultural

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New Delhi-110012, January 16, 1974.

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## SHORT SCIENTIFIC NOTES

### Unit Cell Dimensions and Space Group of $\beta$ - $\text{IrHCl}_2$ [ $\text{AsC}_2\text{H}_5(\text{C}_6\text{H}_5)_2$ ]<sub>3</sub>

Several mono hydrido, octahedral complexes of Iridium of the type  $\text{IrHX}_2\text{L}_3$ , where X is a halogen and L is a tertiary arsine, have been isolated in two isomeric forms  $\alpha$  and  $\beta$ <sup>1,2</sup>. Structures have been assigned to these on the basis of their infrared and nmr spectra<sup>3</sup>. It would be of interest to confirm these structural assignments by X-ray diffraction and locate the correct position of the hydridic hydrogen in these molecules. Further very few such compounds wherein a hydridic hydrogen is directly linked to the metal have been investigated by X-ray methods<sup>4-7</sup>. Hence the X-ray determination of the structure of the  $\beta$ -form of  $\text{IrHCl}_2$ , [ $\text{AsC}_2\text{H}_5(\text{C}_6\text{H}_5)_2$ ]<sub>3</sub> has been taken up. The compound was prepared as reported earlier<sup>2</sup>. Single crystals were grown from methylene dichloride-methoxy ethanol solution of the compound.

The Unit cell dimension and space group of the crystal have been determined using Buerger X-ray Precession camera and  $\text{CuK}\alpha$  radiation. The crystals are monoclinic with the following cell dimensions :

$$\begin{aligned} a &= 13.18 \text{ \AA} & \rho_{\text{mea}} &= 1.67 \text{ gm/cm}^3 \\ b &= 18.94 \text{ \AA} & \rho_{\text{cal}} &= 1.67 \text{ gm/cm}^3 \\ c &= 16.55 \text{ \AA} & Z &= 4 \\ \beta &= 91^\circ \end{aligned}$$

The systematic absences observed are :

$$\begin{aligned} \text{OkO} - k \text{ odd} \\ \text{hol} - h + l \text{ odd} \end{aligned}$$

The absences correspond to the space group  $\text{P}_{21/\text{a}}$ .

Further work on the structure determination is in progress.

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### A Record of Triassic Ostracodes from Kashmir, Himalayas

Poorly preserved ostracodes have been recovered for the first time by the acid etching of dark bluish-grey limestone samples collected from Lower Triassic thin bedded limestones and shales (*Meekoceras horizon*) from Mandakpal, Anantnag District, Kashmir. Ostracodes are represented by five genera : *Bairdia*, *Monoceratina*, *Microcheilinella*, *Judahella* and *Hungarella* which have already been reported from Salt Range, Pakistan (Sohn, 1970)<sup>1</sup>. The ostracode assemblage includes long ranging genera with the exception of *Judahella* which has not been reported from rocks other than the Triassic. The ostracode fauna suggests shallow water environment of deposition. Recently Triassic ostracodes have been reported from Alaska and Nevada<sup>2</sup>, Israel<sup>3</sup>, France, and Austria<sup>4</sup>.

Ostracodes are associated with a number of well preserved conodonts and microgastropods and a few fish remains, mostly teeth and placoid scales. The age of the ostracode-bearing horizon has been determined as *Smithian* to *Spathian* on the basis of biostratigraphically significant conodont species *Neospathodus waageni* Sweet and *Neogondolella elongata* Sweet. Middle Triassic ostracodes have also been discovered from Niti Pass and Kalapani Limestone sequence exposed near Lapthal in Malla Johar, Kumaun Himalayas<sup>5</sup>.

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### Pearl Glands in *Carica papaya* L.

The various types of pearl glands are reported in Ampelidaceae, Begoniaceae, Caesalpinaceae, Moraceae, Piperaceae, Sterculiaceae and Urticaceae<sup>1</sup>. The development and structure of pearl gland is also reported in *Cayratia carnosa*<sup>2</sup> and *Polyalthia longifolia*<sup>3</sup>. As far as we are aware no such glands are reported in *Carica*. And incidentally this is the first report in the family Caricaceae.

In *C. papaya* the pearl glands are found near the ribs of lamina and on the petiole of young and old leaves, on the stem and the flowers. They are also present on small shoots appearing on the main trunk of *Carica*. The glands are small, lustrous and transparent structures appearing in rainy and winter seasons. They are multicellular, tiny spherical or elliptical and easily detachable structures. They are stalked or sessile. When stalked the stalk cells (A, Fig. 1) are smaller than the body cells (B, Fig. 1). Unlike in Vitaceae the glands are without

nuclei. The glands vary from 333  $\mu$ m to 1000  $\mu$ m in height and 226  $\mu$ m to 453  $\mu$ m in diameter.

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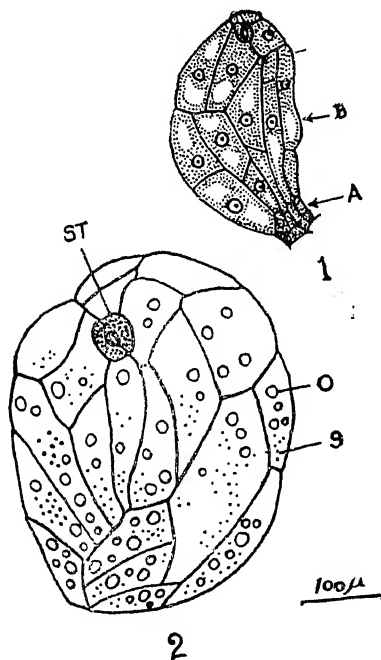
### A Leaf Spot Disease of *Pongamia glabra* Vent. Caused by *Trichothecium* (*Cephalothecium*) *roseum* (Pers.) Link ex Fries.

During the rainy season of 1973 a leaf spot disease of *Pongamia* was observed in the College of Agriculture Campus, KAU, Vellayani. The disease manifested in the form of circular to irregular brown necrotic spots on the young as well as on the mature leaves. The lesions gradually extended and involved a major portion of the lamina. The spots had a light brown centre and a dark margin with a conspicuous yellow halo.

A species of *Trichothecium* was repeatedly isolated in pure culture from the necrotic spots and its pathogenicity was established. The fungus on comparison agreed in all respects with *Trichothecium roseum* which has been recorded as a hyperparasite on *Puccinia graminis tritici* (Pers.) Frikss and Henn. (Sreekantiah and Joshi, 1958). It has been recorded as a parasite on sweet oranges causing fruit rot (Cheema and Jeyarajan, 1972), foliar rot of greenhouse cucumber (McKeen, 1955) and leaf spot of *Kalanchoe pinnata* (Ponnappa, 1967). A perusal of literature indicated that *T. roseum* is a new record on *Pongamia glabra* Vent.

The authors are gratefully thankful to Dr. A. K. Sarbhoj, Curator, I.A.R.I., New Delhi, for the help rendered and to Dr. J. Sam Raj. Dean, KAU, for providing the necessary facilities.

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January 15, 1974.



FIGS. 1-2

epidermis<sup>3</sup> but the stomata are present. There is a single terminal stoma with two guard cells containing granular starch (S, Fig. 2). All the large polygonal cells of the gland appear to be secretory. They contain minute starch grains (S), protein and oil globules (O) as judged by the microchemical tests. These cells have vacuolated cytoplasm and

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## REVIEWS AND NOTICES OF BOOKS

**Bayesian Inference in Statistical Analysis.** By George E. P. Box and George C. Tiao. (Addison-Wesley, Massachusetts), 1973. Pp. xviii + 588. Price \$ 19.60.

This book presents the researches carried out by the authors in cooperation with David Cox, Norman Draper, David Lund, Wai-Yuan Tan, Arnold Zellner. Each of the chapters 2 through 10 is based on the research papers, by these workers, published in the journals like *Ann. Math. Statist.*, *Biometrika*, *J. Amer. Statist. Assoc.*, *J. Roy. Statist. Soc.*, during the years of 1962-68. A good almost-exhaustive long list of these principal references along with other general references is appended at the end of the book.

The use of Bayes' theorem as a basis for statistical inference has been a controversial issue for long and there have been strong opponents as well as supporters of it. But in recent times Bayes' mode of reasoning has found a place of interest in spite of some lingering opposition. As the book reports the research work from the last decade, it has a valuable place among the research workers of this decade. The book can be profitably used for a special two semester graduate course in Bayesian inference, as the prerequisites for studying the book are only the knowledge of elementary probability, usual sampling theory analysis, calculus, and matrix algebra. Sufficient material from the book can even be included in an advanced undergraduate course.

First chapter is devoted to explain the nature and role of Bayesian inference in the general framework of statistical inference, in particular and scientific investigation in general. It considers the choice of prior distributions (specially non-informative ones), the problem of nuisance parameters, and relevance of sufficient statistics. Chapter two forms background to the chapters that follow. Bayesian methods which run parallel to the inferential techniques of usual sampling theory are derived here with equal facility, indeed in better manner sometimes. Chapter three onwards consider certain inferential problems which have not been completely satisfactorily solved by sampling theory. In doing this, the authors say, that they feel that the value of Bayesian analysis may perhaps be judged by considering to what extent it supplies insight and sensible solutions for what are known to be awkward problems. Comparisons between the usual sampling theory results and the corresponding

Bayesian results are made everywhere and form a salient feature of the book.

Practically every chapter has some appendices. Every appendix is devoted to some result(s), which is (are) useful in complete understanding of the subject-matter of the chapter but the derivation and/or discussion of which in the main text might have marred the continuity of thought. At the end of the book there are five sufficiently detailed tables in respect of standard sampling distributions. These include the values of  $F$ ,  $F$ , and  $x^2$ ,  $x^2$  which are not available in the usual text-books. There are separate author and subject indexes. Paper used, printing and get-up of the book is very good.

V. G. TITEKAR.

**Differential Equations Theory and Use in Time and Motion.** By Alice B. Dickinson. (Addison-Wesley Pub. Co. Ltd., Reading, Massachusetts, 01867, U.S.A.), 1972. Pp. ix + 258. Price \$ 10.40.

The above book deals with ordinary linear differential equations of first and second order. The study of motion and the measurement of time are main considerations in this book for studying the physical situations whose mathematical models are the differential equations of the above type.

For an undergraduate student who is not yet exposed to the details of ordinary differential equations but had some training in calculus this book will be helpful. Chapter 1 consists of basic concepts such as function, domain, integral curves, existence and uniqueness of solutions and the solutions of first order linear differential equations. Some good elementary questions which an intelligent beginner can think of such as extension of existence theorem from an open interval to a closed interval have been systematically discussed. Throughout the book firstly physical problems have been considered and then their mathematical models. Most of the physical problems considered are well-known problems such as path of a projectile, Kepler's second law, Newton's second law of motion, motion of a stretched spring, vibrations of a string, etc. The physics as well as mathematics of these problems are discussed in detail. For those who are reading the book at a stretch the author has provided moments of leisure also by giving history of measurement of time, Clepsydra and Carbon-14 dating in Chapter 1; example of resonance in Chapter 2 and

the overtones of the membrane of tabla (a musical instrument) with sketches in Chapter 3.

Chapter 2 deals with the ordinary linear differential equations of second order. The contents are essentially the same which form a small portion of many of the books on engineering mathematics or books on basic mathematics.

Chapters 3 and 4 deal with the series solution of the differential equations and convergence proofs of Picard's iterative method respectively.

The book ends with a note of Euler's method and Runge-Kutta method for numerical solution of differential equations.

As a whole, the reviewer feels that this book does not have enough merit over the other books existing on such topics.

S. C. GUPTA.

## ANNOUNCEMENTS

### Award of Research Degrees

Karnatak University, Dharwar, has awarded the Ph.D. degree in Geology to Shri S. M. Appanagoudar for his thesis entitled "Granite Syenite and Associated Rocks of Koppal Area, Raichur, District, Karnataka State"; Ph.D. degree in Botany to Miss M. Chandani, for her thesis entitled "Some Aspects of Cytology and Cytotaxonomy of the medicinal plants of Sahayadri ranges".

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri J. Rajasekhara Rao, for his thesis entitled "Studies on Polyphenolic Constituents of Indian Medicinal

Plants"; Ph.D. degree in Botany to Shri M. Ramakrishna Rao, for his thesis entitled "Physiological Studies on Dormancy in Groundnut Seeds (*Arachis hypogaea* L.): Metabolic Changes During, After-Ripening and at the Initial Stages of Germination"; Ph.D. degree in Zoology to Shri Md. Basha Mohideen, for his thesis entitled "Some Aspects of Cellular Metabolism in a Euryhaline Fresh Water Fish, Acclimated to Heterosmotic Media".

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Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Shri Anadi Charan Dash, for his thesis entitled "Reactivity and Stability of Metal Complexes"; Ph.D. degree in Botany to Miss Sampuran Kaur, for her thesis entitled "Inheritance of Resistance to Blast Disease in Rice"; Ph.D. degree in Botany to Shri K. Pavithran, for his thesis entitled "Heredity and Environment-factors affecting Phototypic Expression of Hotched Kernel in Rice".

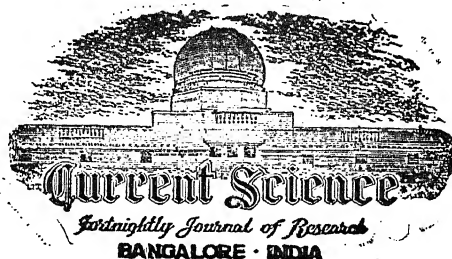
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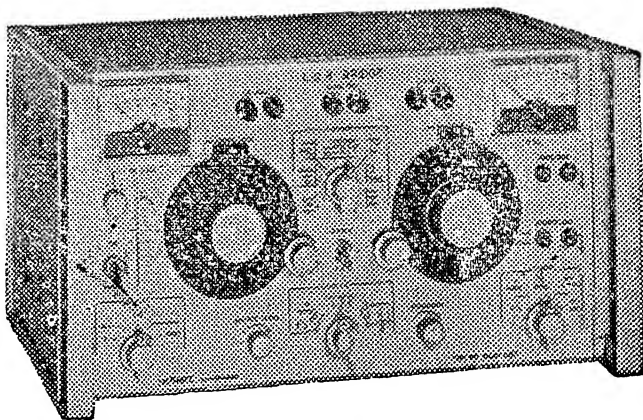
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# TRUE POTENTIAL ENERGY CURVES, DISSOCIATION ENERGY AND $r_e$ -CENTROIDS OF DIATOMIC GeO

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FROM the recent spectroscopic measurements on rotational analysis of some bands of GeO molecule in the  $\lambda$  region (1.0-1.4  $\mu$ ), it is found that the  $r_e$  and  $B_0$  values for  $X^{12}\Sigma^+$  and  $A^{12}\Pi$  ( $2-12$ ) are very different from those of  $X^{12}\Sigma^+$ . Recently, true potential energy curves for the two electronic energies of the ground state of GeO have been calculated by Morse's method using the rotational constants reported by Venkatesh. As an exact knowledge of the potential energy potential energy curves and the  $r_e$  and  $B_0$  values of the species involved in a reaction can be obtained from atomic and molecular constants, an attempt has been made to calculate the potential energy curves and dissociation energies of GeO using the new data reported by Murthy et al. on IBM 1100 computer.

It was chosen that the modified Ritz Method<sup>1</sup> is as effective as RKR method in giving reliable potential energy curves on many cases. Hence, this simple method has been used in evaluating the maximum and minimum turning points in the ground and excited states of GeO molecule. At the same time potential energy curves have also been determined by using Morse empirical potential function to test how far the  $X^{12}\Sigma^+$  ground and excited ( $A^{12}\Pi$ ) states obey Morse potential. It is also known that the difference of the observed rotational constant ( $B_0$ ) from that of the calculated value, according to Pekeris relation, is a measure of deviation from the Morse curve. The calculated and observed values of  $B_0$  and percentage of deviation in the upper and lower states are presented in Table I. As shown in Fig. 1, the true turning

points of the excited state ( $A^{12}\Pi$ ) can be seen from Fig. 2 that the true curve almost coincides with the Morse curve, since the deviation of  $B_0$  is small. The turning points of fifteen vibrational levels of the lower state and eleven vibrational levels in the upper state are presented in Tables II and III along with their  $U_1$  and  $U_2$  values.

TABLE II

True potential energy curves of the ground state of GeO

$v$	$U$ (cm <sup>-1</sup> )	$r_1$ (Å)	$r_2$ (Å)
0	489.6	1.573	1.675
1	1166.8	1.541	1.716
2	2436.4	1.530	1.750
3	3395.5	1.504	1.778
4	4348.5	1.491	1.803
5	5389.4	1.480	1.836
6	6323.1	1.470	1.847
7	7443.3	1.463	1.868
8	8661.1	1.454	1.885
9	8969.5	1.447	1.905
10	9869.5	1.440	1.927
11	10763.7	1.434	1.945
12	11641.0	1.438	1.964
13	12513.5	1.433	1.982
14	13377.5	1.418	2.000
15	14241.7	1.414	2.017

TABLE I

State	$B_0$ observed (cm <sup>-1</sup> )	$B_0$ calculated (cm <sup>-1</sup> )	Deviation in the calculated value
$X^{12}\Sigma^+$	0.0167	0.0070	46%
$A^{12}\Pi$	0.0034	0.0003	9%

points differ appreciably from those of Morse at higher levels. However, the difference is noticeable at the lower levels also when the deviation in  $B_0$  is more as in the ground state ( $X^{12}\Sigma^+$ ). For

Though there are various estimates of dissociation energies proposed by many workers,<sup>2-10</sup> there is still considerable divergence between the thermochemical and spectroscopic values. Hence to estimate the dissociation energy, the true potential energy curves of a molecular electronic state have been used to fitting an empirical potential energy curve to the true potential energy curve. The Hulsburt-Hirschfelder and Lippincott three parameter func-

TABLE III

True potential energy curves of the excited state of GeO

$v$	$U \text{ cm}^{-1}$	$U + T_e \text{ cm}^{-1}$	$r_{\min} (\text{\AA})$	$r_{\max} (\text{\AA})$
0	324.6	38084.3	1.701	1.827
1	967.6	38727.3	1.662	1.881
2	1600.2	39359.9	1.636	1.921
3	2226.7	39986.4	1.616	1.955
4	2844.4	40604.1	1.600	1.986
5	3455.3	41215.0	1.586	2.014
6	4056.9	41816.6	1.573	2.042
7	4650.4	42410.1	1.562	2.068
8	5238.8	42998.5	1.550	2.095
9	5806.6	43566.3	1.541	2.121
10	6389.1	44148.8	1.534	2.142
11	6955.2	44714.9	1.526	2.165

tions have been shown to fit to a good extent to the true potential energy curves. Recently Szőke and Baitz<sup>12</sup> have proposed a potential energy function which is determined by the electronegativity of the atoms constituting the diatomic molecule.

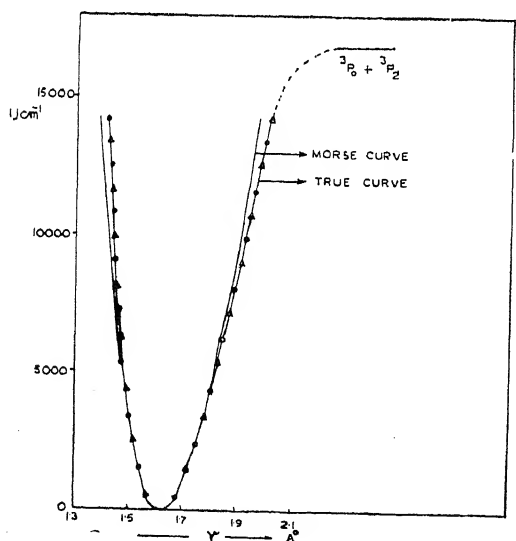


FIG. 1. Potential energy curve of the ground state.  $\Delta$  Electronegativity function.  $O$  Lippincott three parameter function.  $\bullet$  Hulbert-Hirschfelder function.

They have shown that this function also gives striking agreement with RKR curves in the case of alkali metal molecules  $\text{Li}_2$  and  $\text{K}_2$ . Hence in the present case the above three functions have been used for the purpose of determining dissociation energy. It is shown in Figs. 1 and 2, that the three functions give reasonable values for the dissociation energies of the ground and excited states of GeO. These dissociation energies calculated in the ground ( $X^1\Sigma$ ) and upper ( $^1\Pi$ ) states are  $33500 \text{ cm}^{-1}$  (4.153 eV) and  $20000 \text{ cm}^{-1}$  (2.479 eV) respectively.

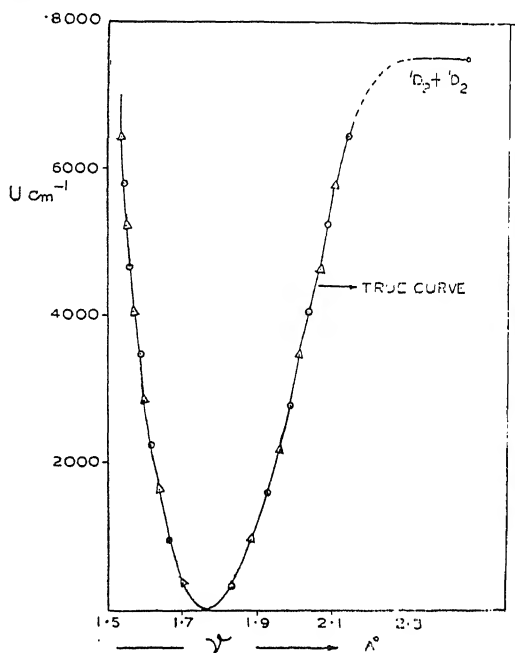


FIG. 2. Potential energy curve of the excited state.  $\Delta$  Electronegativity function.  $O$  Lippincott three parameter function.  $\bullet$  Morse curve.

The dissociation products in the upper and lower states are determined as follows. Since

$\text{Te} + \text{De}'(^1\Pi) = \text{De}''(^1\Sigma) + \text{sum of the atomic excitation energies}$ , the sum of the atomic excitation energies is  $24259.7 \text{ cm}^{-1}$  ( $37759.7 + 20,000 - 33,500 = 24259.7 \text{ cm}^{-1}$ ). This is approximately equal to the sum (23,994) of the atomic excitation energies of  $\text{Ge}(^1D_2 - 7,126 \text{ cm}^{-1})$  and  $\text{O}(^1D_2 - 15,868 \text{ cm}^{-1})$ . Thus the GeO molecule gets dissociated in the upper and lower states as

$\text{Ge}(^1D_2) + \text{O}(^1D_2)$  in the upper state and  $\text{Ge}(^3P_0) + \text{O}(^3P_2)$  in the lower state. So it is more probable that the combination of  $\text{Ge}(^1D_2) + \text{O}(^1D_2)$  is responsible for giving an excited ( $^1\Pi$ ) state of GeO molecule. The dissociation energy  $\text{De}''$  in the ground state of GeO estimated to be

TABLE IV  
r-Centroids of  $A^1\Pi-X^1\Sigma$  system of GeO

$v'/v''$	0	1	2	3	4	5	6
0 a	1.685	1.712	1.739	1.765	1.793	1.820	1.846
b	1.684	1.712	1.739	1.766	1.793	1.819	1.845
c	2659.3	2730.2	2804.2	2882.0	2963.4	3048.4	3137.7
1 a	1.664	1.694	1.721	1.748	1.775	1.802	1.828
b	1.665	1.694	1.720	1.748	1.775	1.801	1.827
c	2614.6	2683.3	2754.8	2829.2	2907.7	2989.7	3075.6
2 a	1.647	1.675	1.703	1.731	1.757	1.783	1.810
b	1.647	1.675	1.703	1.730	1.757	1.783	1.809
c	2575.0	2638.1	2707.6	2779.9	2855.5	2934.3	3017.0
3 a	1.627	1.657	1.686	1.713	1.739	1.765	
b	1.628	1.657	1.685	1.713	1.739	1.766	
c	2531.2	2595.3	2662.5	2732.2			
4 a	1.608	1.638	1.667	1.696	1.722	1.748	
b	1.609	1.639	1.667	1.695	1.722	1.749	
c	2492.2	2554.5	2619.5	2686.7	2757.5		
5 a	1.589	1.619	1.650	1.678	1.705	1.731	
b	1.589	1.620	1.650	1.678	1.705	1.731	
c	2454.7	2515.0	2578.0	2650.4	2711.8		
6 a	1.568	1.600	1.631	1.660	1.688	1.714	
b	1.569	1.601	1.632	1.660	1.688	1.715	
c	2419.0	2476.7	2538.7	2602.2	2668.3	2737.0	
+5	+4	+3	+2	+1	0	-1	-2
0.011	0.011	0.011	0.010	0.010	0.009	0.009	0.009

$\bar{r}_{v', v''}$  values in Å U. (a) graphical method, (b) quadratic equation method, (c) wavelength of the band heads in Å U.

33500 cm<sup>-1</sup> (4.153 eV) is considerably lower than the value (6.9 eV) reported by Gaydon.

#### r-CENTROIDS

The  $\bar{r}$ -centroid ( $\bar{r}_{v', v''}$ ) representing the characteristic internuclear separation of a  $v' \rightarrow v''$  transition in a diatomic molecular band system, has been defined by Nicholls and Jarman<sup>13</sup> as

$$\bar{r}_{v', v''} = \frac{\int \psi_{v'} r \psi_{v''} dr}{\int \psi_{v'} \psi_{v''} dr}$$

where  $\psi_{v'}$  and  $\psi_{v''}$  are the vibrational wave functions and  $r$  is the internuclear distance. In the present work, both the graphical and quadratic equation methods (Nicholls and Jarman) have been employed for the evaluation of  $\bar{r}$ -centroids for the bands of  $A^1\Pi-X^1\Sigma$  system of GeO molecule. The results are presented in Table IV. A smooth curve has been obtained when a graph was drawn between  $\bar{r}_{v', v''}$  and  $\lambda_{v', v''}$ . The sequence difference  $\Delta r = r_{v'+1, v''+1} - \bar{r}_{v', v''}$  for this system is found to be approximately constant as shown in Table IV.

It is expected that the true potential energy curves presented here will be useful in computing Franck-Condon factors and transition probabilities.

The authors are grateful to Prof. B. R. Rao, Head of the Department of Physics and Director, Computer Centre, Andhra University, Waltair, for granting financial assistance towards computing charges.

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# FORMATION CONSTANTS OF CHELATES OF 7-iodo-8-HYDROXY QUINOLINE-5-SULPHONIC ACID WITH YTTRIUM AND SOME LANTHANIDES

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## ABSTRACT

Formation constants and thermodynamic parameters of chelation  $\Delta F$ ,  $\Delta H$  and  $\Delta S$  for chelates of 7-iodo-8-hydroxy-quinoline-5-sulphonic acid with yttrium, lanthanum, neodymium, gadolinium and dysprosium are reported.

7-iodo-8-hydroxy quinoline-5-sulphonic acid was first introduced by Yoe<sup>1</sup> for the colorimetric estimation of  $Fe^{3+}$ . Subsequent investigations were confined to the use of the reagent for chelometric studies with some divalent<sup>2,3</sup> and trivalent<sup>4</sup> metal ions. Recently Chang *et al.*<sup>5</sup> have determined the formation constants of a number of rare earth chelates of the reagent at 25° C using water dioxane medium.

Literature survey has indicated absence of information on thermodynamic parameters of chelation on rare earth-7-iodo-8-hydroxyquinoline-5-sulphonic acid chelates. The present work hence deals with data obtained on formation constants of chelates of  $Y^{3+}$ ,  $La^{3+}$ ,  $Nd^{3+}$ ,  $Gd^{3+}$  and  $Dy^{3+}$  with 7-iodo-8-hydroxy quinoline-5-sulphonic acid, at 30°, 40° and 50° C at fixed ionic strength. Values of thermodynamic parameters for chelation are also reported.

## EXPERIMENTAL

**Metal ion solutions.**—All rare earth metal ion stock solutions were prepared by dissolving appropriate quantities of rare earth chlorides (obtained from the respective rare earth oxides, purity 99.9%, supplied by M/s. Indian Rare Earths, Kerala, India) in about 0.1 M perchloric acid (Riedel, Germany), as to give a stock solution with metal ion content lying between  $1.2 \times 10^{-3}$ – $1.6 \times 10^{-3}$  M. Metal ion solutions were analysed for the metal ion content as per usual procedure<sup>6</sup>.

**Ligand solution:** Ligand stock solution was about  $6 \times 10^{-3}$  M and obtained by dissolving requisite quantity of 7-iodo-8-hydroxy quinoline-5-sulphonic acid (Fluka, Swiss, recrystallised, m.p. 259–260° C, lit. 260° C, equivalent wt. ( $-SO_3H$ ): 351.1) in freshly boiled double distilled water.

**Alkali solution:** Carbonate free sodium hydroxide solution of about 1.5 M strength was used in all titrations. This solution was standardised against solid potassium hydrogen phthalate (BHD AnalaR) before every titration.

**Sodium perchlorate solution:** Standard sodium perchlorate solution was obtained by neutralising standard perchloric acid solution with sodium hydroxide. The strength of sodium perchlorate was adjusted so as to help in maintaining an ionic

strength of 0.1 in the solutions A, B and C prepared below. This was necessary as the perchloric acid used in the metal ion solution was not always of the same strength.

**Titration Technique.**—The Calvin-Bjerrum titration technique as adopted by Irving and Rossotti<sup>7</sup> was used. Three sets of solutions for each metal ion were prepared for studies at any given temperature.

Solution A : 25 ml  $HClO_4$  (0.1 M) + 50 ml  $H_2O$  + 25 ml  $NaClO_4$

Solution B : 25 ml  $HClO_4$  (0.1 M) + 50 ml ligand solution + 25 ml  $NaClO_4$ .

Solution C : 25 ml  $HClO_4$  (0.1 M) containing metal ion + 50 ml ligand solution + 25 ml  $NaClO_4$ .

In all experiments the ligand to metal ratio was kept above 5.

pH titrations at any required temperature were achieved by placing the solutions in a glass thermostat ( $\pm 0.1^\circ C$ ) and adding sodium hydroxide (0.01 ml aliquot) the solution being stirred by a glass stirrer mechanically. Nitrogen gas (M/s. Indian Oxygen Ltd., Bombay) presaturated with water vapour at the same temperature was bubbled

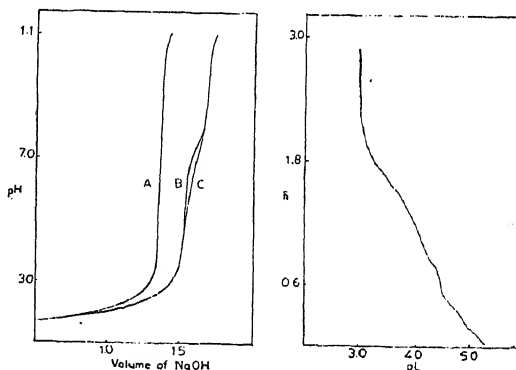


FIG. 1.  $La^{3+}$ -7I8HQ5S system; 30° C;  $\mu = 0.1$ . through the solution during titration. A Philips pH meter, No. PR 9405 (accuracy  $\pm 0.05$ ) was used, and was standardised daily at each temperature using phthalate buffer<sup>8</sup>. All titrations were performed in duplicate to test for reproducibility.

Values of  $\bar{n}_A$ ,  $\bar{n}$  and  $pL$  for the metal ion-ligand titrations were obtained from graphs corresponding to titrations of solutions A, B and C (Fig. 1 representative titration curve for  $\text{La}^{3+}$ ) and were calculated using the following expressions<sup>7</sup>.

$$\bar{n}_A = \left\{ \frac{1}{V} T_L^0 + \frac{(N+E^0)(V'-V'')}{(V_0+V')} \right\} / T_L^0$$

$$\bar{n} = \frac{(V''-V') [N+E^0+T_L^0(V-\bar{n}_A)]}{(V_0+V'') \bar{n}_A T_m^0}$$

and

$$pL = \log \left[ \frac{1+K_1(H^+)+K_1K_2(H^+)^2}{T_L^0-\bar{n} T_m^0} \cdot \frac{V_0+V''}{V_0} \right]$$

Values of proton ligand stability constants of 7-iodo-8-hydroxy quinoline-5-sulphonic acid were treated by (i) interpolations at half  $\bar{n}$  values and (ii) interpolation at various  $\bar{n}_A$  values at each temperature and the mean reported. Values of metal ligand stability constants  $\log K_1$ ,  $\log K_2$  and

meters  $\Delta F$ ,  $\Delta H$  and  $\Delta S$  for chelation calculated as per standard procedure.

#### RESULTS AND DISCUSSION

Values of  $\bar{n}_A$  did not go beyond 1.8 at the three temperatures indicating presence of only two dissociable protons in the ligand. Proton ligand stability constants (for  $-\text{OH}$  and  $\text{NH}^+$  formation) are in agreement with those reported earlier<sup>3</sup>.

Values of  $\bar{n}$  for metal, in no case went beyond 2.9 indicating formation of 1:3 complex between metal and ligand. Stepwise formation constants for the complex  $\log K_1$ ,  $\log K_2$  and  $\log K_3$  reported in Table I show trends similar to those observed earlier for 8-hydroxy quinoline-5-sulphonic acid chelates with rare earths<sup>9</sup>. In comparison the chelates of 7-iodo-8-hydroxy quinoline-5-sulphonic acid are weaker than the corresponding chelates of 8-hydroxyquinoline-5-sulphonic acid, which may be ascribed to the lower basicity of the 7-iodo derivative. In all cases the nature of the metal titra-

TABLE I

Chelate formation constants and thermodynamic functions of yttrium and some lanthanides with 7-iodo-8-hydroxy quinoline-5-sulphonic acid

Ionic Strength :			$\mu = 0.1$		Temp. : 30° C	
Metal ion $n$ in $K_n$		$\log K_n^*$	$-\Delta F$ kcal/mole	$-\Delta H$ kcal/mole	$+\Delta S$ cal/mole	
$\text{H}^+$	1.	6.89	9.55	4.80	15.6	
	2.	2.40	3.30	1.80	4.9	
$\text{Y}^{3+}$	1.	5.80	8.04	2.23	19.1	
	2.	4.96	6.87	2.45	14.6	
	3.	4.08	5.65	2.90	9.0	
$\text{La}^{3+}$	1.	4.52	6.26	1.56	15.3	
	2.	3.64	5.04	3.34	5.6	
	3.	3.00	4.16	2.90	4.1	
$\text{Nd}^{3+}$	1.	5.01	6.94	2.45	15.1	
	2.	4.10	5.68	2.45	10.6	
	3.	3.02	4.18	2.90	4.2	
$\text{Gd}^{3+}$	1.	5.34	7.40	4.24	10.4	
	2.	4.42	6.13	2.68	11.3	
	3.	3.21	4.45	3.57	2.9	
$\text{Dy}^{3+}$	1.	5.17	7.17	1.34	19.2	
	2.	4.11	5.70	3.12	8.5	
	3.	3.00	4.16	3.35	2.6	

\* Accuracy of  $\log K$  values was  $\pm 0.05$  for the first step and  $\pm 0.07$  for the second and third steps respectively.

$\log K_3$  obtained from  $\bar{n}$  vs  $pL$  curves (Fig. 1) were refined both by (i) correction term method and (ii) graphical method and average of these reported. The results are reported in Table I. Table I also gives values of thermodynamic para-

tion curves served to indicate absence of hydrolysis of the metal ion.

Thanks are due to Dr. A. Gopalakrishna, Director, Institute of Science, Nagpur and Dr. V. V. Deshpande, Professor and Head of the Department of

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## EFFECTS OF NYMPHAL TREATMENT WITH TEPA ON REPRODUCTION OF *DYSDERCUS KOENIGII* F.

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### ABSTRACT

Varying doses of tapa applied topically on the different nymphal instars of *Dysdercus koenigii* F. produced adults which exhibited a remarkable degree of sterility. The effects of this treatment on the mortality and the formation of abnormal stages of development have also been investigated. 100% sterility has been achieved with a minimum dose of 0.5 µg of tapa/nymph in the third and fourth instars.

### INTRODUCTION

**R**ED cotton-bug *Dysdercus koenigii* F. is an important pest of cotton and some vegetable crops in India. Control with insecticides in the fields has been reported but with limited success. The encouraging results of the use of chemosterilants in different groups of insects<sup>1,2,7,9,10</sup> have created an interest in using such chemicals as a possible means of control. Before determining the feasibility of the use of such chemicals in fields for a successful control, laboratory investigations are of utmost importance. Some information regarding the sterilization of a number of species of bugs is available in the literature. Economopoulos and Gordon (1969) have induced sterilization in male *Oncopeltus fasciatus* (Dallas) with tapa. Carcavallo and Carabajal (1971) carried out sterilization experiments on three species of *Triatoma*. Economopoulos (1971) has investigated the effects of tretamine on fourth and fifth instars of *O. fasciatus* male nymphs and female adults. Mustafa and Naidu (1964) demonstrated sterilization of male *D. cingulatus* with apholate as a surface-contact sterilant. Degeneration of the oocytes and inhibition of ovarian growth were induced in the adult female *D. cingulatus* with tapa injections<sup>12</sup>. In these investigations of chemosterilization of *Dysdercus*, the chemosterilant compound was administered either orally with the diet or by injection, and the insect was treated in the adult stage.

In our investigations on the effects of tapa on the fecundity and sterility of *Dysdercus*, we have treated both the male and female insects. The chemical has been administered topically. Attempts have been made to find out the most susceptible stage in the life-cycle with a minimum of mortality. Therefore, we selected the nymphal instars where the topical treatment in solution would least affect their general activity, with the result, we had to avoid first two instars because of their very small size. This paper, the first in a series, presents the results of the laboratory tests which give quantitative information on the toxic and sterilizing activity of tapa against *Dysdercus*.

### MATERIALS AND METHODS

Stock culture of *Dysdercus* was maintained in the laboratory at  $27 \pm 2^\circ \text{C}$  in  $22 \times 16 \times 16$  cm glass toffee-jars, covered with muslin held in place by rubber bands. The jars contained food, i.e., dry cotton seeds (previously washed in running water and dried with clean filter-paper or cotton wool) and water (cotton wool dampened with distilled water) in small petri dishes. The experiments were performed on various nymphal stages picked up from the culture within 24 hours of their emergence. Before treatment the insects were immobilized by momentary cooling in the freezing chamber of a refrigerator. A stock solution of tapa, *Tris*(1-aziridinyl) phosphine oxide, was made in acetone. A glass all-glass syringe fitted with micro-

meter was used for delivering the chemical on all the stages which were treated topically with graded concentrations of tapa in 1.0  $\mu$ l drops that were applied the ventral abdominal surface. First and second instar nymphs were not able to tolerate the drops of 1.0  $\mu$ l. Therefore, the treatment was restricted to the third, fourth and fifth nymphal instars only, the stages when the maturation of the gonads actually takes place. The treated insects were removed to the jars containing cotton seeds and soaked cotton pads. They were observed daily for any mortalities or moults during development and these were regularly recorded. In the fifth nymphal stage they were sexed as far as possible and the males and females were separately placed. 3-4 days after adult emergence they were allowed to mate with treated or untreated insects

## RESULTS AND DISCUSSION

### Toxicity and Mortality

The toxicity pattern of four doses of tapa on third, fourth and fifth nymphal instars is given in Table I. The first and second instar nymphs showed a heavy mortality rate, even with 1.0  $\mu$ l of the solvent, acetone/nymph. Therefore, the effectiveness of the chemosterilant on these stages is not considered at present. 0.5-1.0  $\mu$ g tapa/nymph shows a comparatively low mortality rate but the higher doses of 1.5  $\mu$ g and 2.0  $\mu$ g/nymph are quite toxic, especially to the third and fourth instars. The mortality goes considerably high (98.2%) in the fourth instar. The mortality in the fifth instar treatment does not seem to be very high. This is possibly because it enters the adult

TABLE I

Mortality of *D. koenigii* nymphs during development with varying doses of tapa

Stage treated	Dosage of tapa/ nymph ( $\mu$ g/ $\mu$ l acetone)	Mortality in various nymphal instars			Total mortality (out of 60)	Corrected total mortality* (%)
		3rd	4th	5th		
3rd instar	0.0 Control	2	1	0	3	..
	0.5	4	0	5	9	10.52
	1.0	1	0	9	10	12.32
	1.5	21	22	6	49	79.68
	2.0	15	26	10	51	84.21
4th instar	0.0 Control	..	2	0	2	..
	0.5	..	3	1	4	3.41
	1.0	..	15	6	21	32.71
	1.5	..	40	7	47	77.64
	2.0	..	51	8	59	98.24
5th instar	0.0 Control	..	0	0	0	..
	0.5	..	..	4	4	6.70
	1.0	..	..	20	20	33.33
	1.5	..	..	18	18	30.00
	2.0	..	..	26	26	40.00

\* Using Abbot's formula.

of the opposite sex for different experimental designs. Daily examination for the eggs was made and the eggs from the jars were removed within 12-18 hrs of laying. After counting, the eggs were incubated in small vials (2.5  $\times$  5 cm) covered with cotton plugs and kept in the humidity chamber at 27 $\pm$  2° C. The number hatched was determined and recorded. In each test, six replicates of ten insects each, were taken at the start of the treatment, while a control replicate in each test was treated with solvent.

stage sooner than the other two earlier instars. In all these cases, after the imaginal stage is reached, the mortality rate becomes normal and at par with that in control. The total mortality in the third instar treatment is highest because it takes a longer period to reach the adult stage. It is also possible that since same amount of tapa was applied on all the nymphs, the younger stages, being smaller in size, received a greater dose per unit body weight thus leading to higher mortality. X-irradiation of an instar of *D. koenigii* during



its late phase has no effect on its next moult but irradiation during the early stages did affect the next moult<sup>8</sup>. It was therefore suggested that a treatment at a late stage is not very critical for an approaching moult. The present situation could also be explained in this context. Very little mortality is observed in 5th instar treatment but the nymphs treated earlier show more mortality. This is because of the toxic effect being carried over to the next instar. A similar explanation for higher mortality in *Oncopeltus* nymphs treated with tretamine in the early 4th instar than those in fifth, has been put forward<sup>5</sup>. Figure 1 gives a comparative account of corrected total mortality in the three stages treated with four different doses of tapa.

#### Abnormal Stages

As a result of this treatment in all the tests, some of the fifth instar nymphs, instead of moulting into adults, produced abnormal stages of development which were identified as sixth-instar nymphs and juvenile adults. Some nymphs struggled to moult, with old cuticle unsuccessfully cast off and crumpled wings. The results of such treatments are summarised in Table II. The fourth instar nymphs treated with 1.0  $\mu\text{g}$  tapa/nymph produced maximum number of abnormal stages. All these stages died after a week's time, without any reaching maturity. These forms were also allowed to mate with untreated virgin males and females but were found incapable of reproduction. A variety of abnormal stages of development were observed in *D. fasciatus* by treating nymphal instars with

juvenile hormone analogue, methyl fornesoate dihydrochloride, applied topically<sup>4</sup>. In the present study, the formation of abnormal stages could be attributed to some hormonal imbalance caused by the application of the chemosterilant.

TABLE II  
Abnormal stages of development produced in  
*Dysdercus hy tapa treatment*

Stage Treated	Dosage of tapa/ nymph ( $\mu\text{g}/\mu\text{l}$ acetone)	No. of 6th instar/Juvenile; adult produced; (out of 60)	Per cent
3rd instar	0.5	..	..
	1.0	4	6.67
	1.5	4	6.67
	2.0	2	3.34
4th instar	0.5	2	3.34
	1.0	10	16.67
	1.5	6	10.0
	2.0	..	..
5th instar	0.5	..	..
	1.0	3	5.0
	1.5	2	3.34
	2.0	5	8.33

#### Fecundity and Sterility

Because of high mortality in the third instar with dosages of 1.5 and 2.0  $\mu\text{g}$ /nymph, the mating experiments were not done. The adults, surviving from the third instar nymphs treated with 0.5 and 1.0  $\mu\text{g}$ /nymph, were allowed to mate both with untreated and treated virgins of the opposite sex. The data presented in Table III indicates that both the doses cause no hatch in the eggs laid with any of the three crosses, i.e., treated females  $\times$  normal males, treated females  $\times$  treated males and normal females  $\times$  treated males. 0.5  $\mu\text{g}$  dose had also caused a significant reduction in oviposition, which was still more pronounced with 1.0  $\mu\text{g}$  dose. A female treated with 0.5  $\mu\text{g}$  laid an average of 58 eggs which was further reduced to 14.5 eggs female with 1.0  $\mu\text{g}$  dosage. In the control the number of eggs laid was 500/female. A similar reduction in the fecundity was obtained in the adults which emerged from the fourth instar nymphs treated with same doses (Table IV). 0.5  $\mu\text{g}$  of tapa applied to fourth instar nymphs caused 95% sterility if crosses were made between treated male and normal/treated female. In a reciprocal cross between treated females and normal males, the per cent hatch is much higher, showing that tapa induces more sterility in males. A dose of 1.0  $\mu\text{g}$ /nymph shows a similar result. A treated female crossed with a normal male produces on the average 5.4% viable eggs while 100% sterility is achieved in crosses between treated males and normal/treated females.

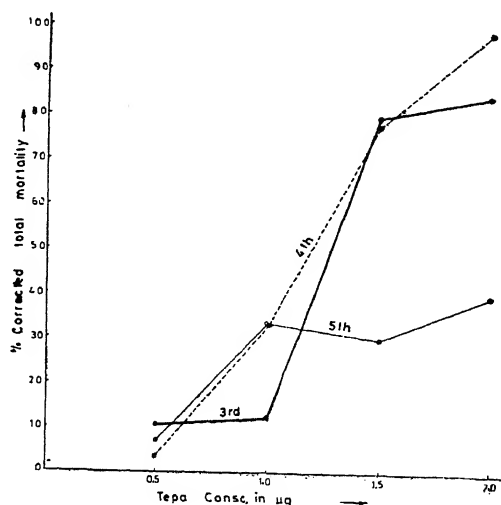


FIG. 1. Mortality curves of third, fourth and fifth nymphal instars treated topically with different concentrations of tapa.

TABLE III

Effect on fecundity, egg hatchability and sterility of *Dysdercus* third instar nymphs topically treated with varying doses of tapa

Treatment dose ( $\mu\text{g}/\mu\text{l}$ acetone/nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0.5	Female	5	290	58	0	0	100
	Male	5	1,635	327	0	0	100
	Both	4	190	475	0	0	100
1.0	Female	4	70	175	0	0	100
	Male	5	575	115	0	0	100
	Both	4	58	145	2	3.45	95.73
	None (Control)	5	2,620	524	2,120	80.92	..

TABLE IV

Effect on fecundity, egg hatchability and sterility of *Dysdercus* fourth instar nymphs topically treated with varying doses of tapa

Treatment dose ( $\mu\text{g}/\mu\text{l}$ acetone/nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0.5	Female	6	1,376	229.3	612	44.47	47.43
	Male	5	2,128	425.6	251	11.79	86.06
	Both	6	338	56.3	42	12.5	85.22
1.0	Female	5	1245	249	67	5.38	93.64
	Male	4	1,174	293.5	0	0	100
	Both	5	240	48	0	0	100
	None (Control)	5	2,540	508	2,160	84.60	...

TABLE V

Effect on fecundity, egg hatchability and sterility of *Dysdercus* fifth instar nymphs topically treated with varying doses of tapa

Treatment dose ( $\mu\text{g}/\mu\text{l}$ acetone/nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0.5	Female	5	1,430	286	534	37.29	53.38
	Male	4	1,144	286	77	6.76	91.55
	Both	4	534	113.5	14	2.6	96.75
1.0	Female	6	654	109	202	30.88	61.4
	Male	5	875	175	0	0	100
	Both	5	410	82	0	0	100
1.5	Female	5	165	33	30	18.18	77.27
	Male	5	1,980	396	40	2.02	97.47
	Both	6	240	40	0	0	100
2.0	Female	4	208	52	16	7.68	90.4
	Male	4	884	221	0	0	100
	Both	5	90	18	0	0	100
	None (Control)	5	2,715	543	2,172	80	..

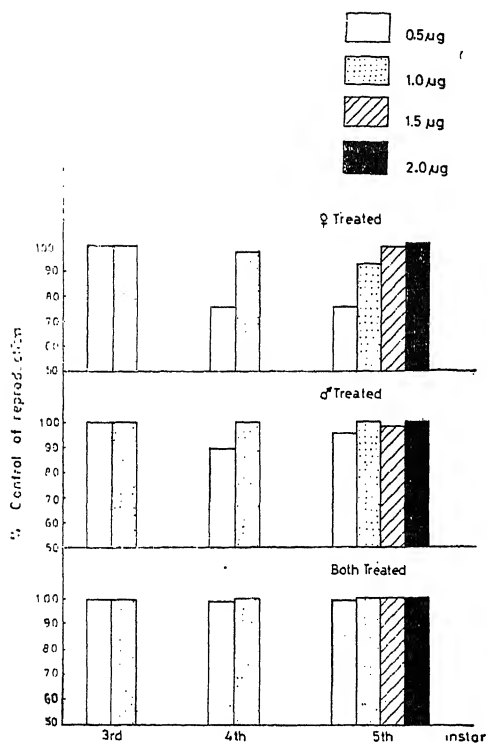


FIG. 2. Results showing per cent control of reproduction in different crosses between the adults derived from the nymphs treated with varying doses of tepa.

The effects of four doses of tepa on the fecundity and egg hatchability from different crosses of adults emerged from the nymph treated in fifth instar are given in Table V. Treated females with 0.5 µg and 1.0 µg/nymph crossed with normal males show 53.4% and 61.1% of sterility respectively. Similar crosses treated with 1.5 µg and 2.0 µg/nymph show 90% sterility. 100% sterility, however, was achieved with 1.0–2.0 µg doses in reciprocal crosses between treated males and normal/treated females. The oviposition rate was also considerably lowered in the crosses between both the treated sexes.

Figure 2 gives a comparative result of the per cent control of reproduction of the adults derived from the stock of nymphs administered with different doses of tepa in different instars after appropriate

crosses. Third nymphal instar treatment gives 100% sterility with tepa dosage as low as 0.5 µg/nymph of either sex. In fourth instar a dose of 1.0 µg/nymph causes 100% sterility in male but slightly less in females. Further 100% sterility in males is produced in the fifth instar treatment with 1.0 µg/nymph. In adult *D. cingulatus* a contact-dose as high as 1.4 mg of apholate/sq inch with an exposure time of four hours could induce 0% hatch<sup>11</sup>. Similarly 94.6% sterility was induced in some insects injected with 5.0 µg of tepa/adult female<sup>12</sup>. It is, therefore, suggested that a low dose of tepa administered to the third and fourth nymphal instars is better to induce successfully a 100% sterility in *Dysdercus* than a very high dosage to the adult.

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## LETTERS TO THE EDITOR

### METASTABLE SOLID SOLUTIONS IN LEAD-TIN ALLOYS

MUCH work<sup>1-3</sup> has been done on germanides and tellurides of gold and silver, nickel-base alloys with silicon, tin and germanium<sup>4</sup>, copper-silver<sup>5</sup>, copper-rhodium<sup>6</sup>, aluminium-magnesium<sup>7</sup>. In the present investigation, we have succeeded for the first time, in preparing lead and tin alloys upto 30 atomic per cent as one of the constituents, from the melt.

For preparing these alloys, appropriate amounts of lead and tin (A.R. grade) were mixed and the mixtures were heated in evacuated silica tubes at 450° C in an oven for four days. At the end of this period the melts were rapidly quenched in ice-cooled water. It was found that the weight losses after quenching were less than 0.12 and therefore, the actual percentage composition of the alloys formed can be assumed to be the same as it was in the original mixture.

TABLE I

Lattice parameters\* of metastable solid solutions in lead-tin alloys

Amount of solute (pb) atomic per cent	Phase† detected	Lattice Parameters		<i>c/a</i>
		<i>a</i> (Å)	<i>c</i> (Å)	
5	tetragonal	5.9503	3.3551	0.5638
10	„	5.9950	3.3857	0.5648
15	„	6.0251	3.4300	0.5691
20	„	6.0496	3.4511	0.5704
25	„	6.1004	3.4849	0.5712
30	„	6.1325	3.5204	0.5740

\* Lattice parameters '*a*' and '*c*' are measured at room temperature.

† The structures of these alloys were determined by 114.6 mm Debye-Scherrer Camera.

The compositions of the alloys investigated and their structure are summarized in Table I. The reported value<sup>8</sup> of the maximum solubility of lead in tin is 1.5 atomic per cent of lead at 183° C. In our case, however, the solubility of lead is considerably increased. Solubility limits<sup>9-10</sup> can be increased beyond the equilibrium conditions if the

rate of the cooling is increased. In the present study, the high rate of cooling has been obtained by quenching the sample in ice-cooled water. It is well known<sup>8</sup> that the solute atoms with larger atomic radii than those of solvent atoms which they replace, tend to increase the lattice parameters with the increase of the solute atoms. In our case, the lattice parameters '*a*' and '*c*' should increase as the solute atoms (lead atoms) have larger radii than the solvent atoms (tin atoms). Our observation that the '*c/a*' ratio increases with the increase in the lead content, means that the relative increase in '*c*' is greater than that in '*a*'.

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### URIDINE-(5') PHOSPHATE : STRUCTURE AND CONFORMATION

WE have been interested in the stereochemistry of nucleic acid constituents and have now obtained the crystal and molecular structure of Uridine-5' phosphate, a disodium salt (5'-UMP. Na<sub>2</sub>). Crystals of 5'-UMP.Na<sub>2</sub> were grown by slow diffusion of acetone into the aqueous solution of the compound. The crystals belong to orthorhombic system with unit cell parameters *a* = 22.88, *b* = 8.877, *c* = 19.592 Å.

The space group is  $C22_1$ , and measured density (1.63 gm/cc) indicated that there are seven molecules of water in the asymmetric unit. Three-dimensional intensity data were collected on a General Electric XRD-490 diffractometer using  $\text{CuK}\alpha$  radiation by stationary crystal, stationary counter method. The structure was solved by two independent approaches, (1) by Patterson, iterative structure factor and electron density calculations and (2) by tangent formula application. Positional and thermal parameters were refined by block diagonal least square calculations, the final reliability index  $R$  being 14.5%. The projection of the molecule viewed down  $b$ -axis is shown in Fig. 1.

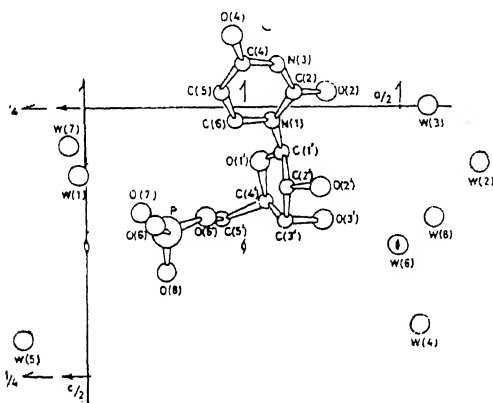


FIG. 1. Projection of the molecule 5'-UMP down  $b$ -axis. Both Sodium atoms and water oxygens are designated as W's as the Na atoms could not be unambiguously inferred because of high temperature factors.

The calculation of the torsion angle for the rotation about the glycosidic bond  $\text{C}(1')\text{--N}(1)$  shows that the base is in the usual *anti* conformation, which is considered energetically more favourable. Least square computations for various four atom combinations of the ribose moiety show the best plane is defined by  $\text{C}(1')\text{--C}(3')\text{--C}(4')\text{--O}(1')$  with  $\text{C}(2')$  displaced by 0.52 Å on the same side as  $\text{C}(5')$ , a conformation usually referred to as  $\text{C}(2')\text{--endo}$ . The conformation about the  $\text{C}(4')\text{--C}(5')$  bond is *gauche-gauche*.

The pyrimidine ring is essentially planar. There is a partial overlap of the bases related by a two fold axis parallel to  $a$ . The molecules are packed as two parallel chains running parallel to  $c$ -axis, with the sodium atoms and water molecules situated in between the two chains.

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## VISIBLE SPECTRA OF THE MOLECULE— $\text{CuCl}$

BAND spectrum of the diatomic molecule- $\text{CuCl}$  has been studied in the past in various details by different workers. Kien<sup>1</sup> was probably, the first to observe bands due to this molecule in bunsen flame. Later on, Harnack<sup>2</sup> using chlorine gas flame and Strutt and Fowler<sup>3</sup> employing active nitrogen tubes, photographed a few red degraded bands in the near ultraviolet region and classified them to form a system, now called the F-x system. In 1927, Ritsch<sup>4</sup> investigated the absorption spectrum of this molecule in wavelength region  $\lambda\lambda$  4000–5506 which he classified into five different systems. He, however, could not record any bands below 3999.65 Å, which were previously observed in emission. Bloomenthal<sup>5</sup> excited  $\text{CuCl}$  molecule in active nitrogen source and identified about twenty-one bands in the spectral region  $\lambda\lambda$  3789–4176 forming the F-x system. Gaydon<sup>6</sup> made a comparative study of intensity distribution for the different visible band systems of this molecule in a cool flame. Sinha<sup>7</sup> also recorded few bands in flame and fitted them in the E-x system. In 1961 employing a high frequency source, Rao and Brady<sup>8</sup> recorded the F-x system, though with a poor intensity, along with other five known systems in the visible region. In addition to a number of new bands in each systems, these authors also observed vibrational isotopic effect in many of the bands. Recently, the thermal emission and absorption spectra of the F-x system for this molecule have been studied by the authors<sup>9</sup>.

The present communication presents an account of the author's findings on the thermal emission spectra studied for the first time of the first five systems in the visible region. Self absorption phenomenon has also been recorded for many of the bands of the D-x and E-x systems.

A small quantity of pure cuprous chloride ( $\text{Cu}_2\text{Cl}_2$ -E. Merck, Darmstadt, Germany) was put in the experimental tube of the high temperature graphite tube furnace. The spectra were excited at about 2200° C in the atmosphere of argon at about 60 torr. Exposures of about two to six minutes were found sufficient to obtain nice photographs on Ilford HP-3 panchromatic plates using Hilger (E-492) large quartz spectograph.

The bands recorded in the spectral region  $\lambda\lambda$  3931–5627 were found, irrespective of their band systems, to be single headed and red degraded. They were classified into five known systems having ground state as the common lower electronic state. While no additional information could be added in the first three systems, viz., A-x, B-x and C-x, recorded in thermal emission, a number of new bands have been identified for the two overlapping

systems, viz., D-x and E-x ( $\lambda\lambda$  3931–4662). These bands along with their vibrational analyses are listed in Table I. The various vibrational constants obtained for the different electronic systems are given in Table II.

It is found that self-absorption phenomenon takes place below 4492 Å. This is why only few bands on the larger wavelength side of the D-x and E-x systems could be observed in thermal emission but others on the shorter wavelength side are absorbed. The bands of the sequences (0, 2), D-x system and (0, 2) and (0, 3), E-x system are found to be diffuse due to both, emission and absorption, tendencies.

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#### ISOLATION OF (+) GALLOCATECHIN FROM CASHEWNUIT SHELLS

THE cashewnuts (*Anacardium occidentale* Linn.) were earlier examined for their chemical constituents. The cashewnut shell liquid (known as CNSL) was shown to contain a number of alkenyl phenols<sup>2,4-8</sup> and phenolic acids<sup>1,3,5,7</sup>. Ethyl gallate<sup>9</sup>, (+) catechin and (–) epicatechin<sup>10</sup> were reported from cashewnut flowers and testa respectively. But the CNSL free shells were not examined so far. During our examination, the methanolic extract of the CNSL free shells (1.5 Kg) was fractionated into ether soluble (yield: 12 g) and ether insoluble (yield: 45 g) fractions.

The ether soluble fraction is a yellow liquid which was filtered through silica gel column (75 g) and washed with plenty of ether (5 litres). Distillation of the ether solution furnished pale yellow crystalline solid (yield 10 g) which was crystallised fractionally from benzene, chloroform and ether. Benzene gave exclusively syringic acid (yield 3.5 g) while ether afforded gallic acid (yield: 3 g; m.p. 235°). Chloroform gave a mixture of these

TABLE I

New band heads for the D-x and E-x systems of the molecule-CuCl

$\lambda$ in Å air	$\nu$ in $\text{cm}^{-1}$		Vibrational analysis ( $v', v''$ ) syst.
	obs.	calc.	
3931.4	25429	25430	(6, 0) E-x
3957.1	25264	25264	(11, 5) E-x
4193.5	23840	23846	(11, 8) D-x
4198.2	23813	23807	(12, 9) D-x
4206.5	23766	23773	(7, 5) E-x
4209.7	23748	23749	(8, 6) E-x
4266.4	23432	23426	(4, 3) E-x
4270.6	23409	23409	(5, 4) E-x
4274.1	23390	23392	(6, 5) E-x
4278.3	23367	23374	(7, 6) E-x
4282.2	23346	23352	(8, 7) E-x
4283.4	23339	23336	(9, 8) E-x
4310.7	23192	23191	(7, 6) D-x
4317.5	23155	23162	(8, 7) D-x
4322.4	23129	23131	(9, 8) D-x
4338.1	23045	23048	(2, 2) E-x
4354.7	22957	22959	(8, 8) E-x
4356.4	22948	22945	(9, 9) E-x
4376.2	22844	22846	(5, 5) D-x
4378.9	22830	22821	(6, 6) D-x
4421.9	22608	22608	(5, 6) E-x
4423.5	22600	22596	(6, 7) E-x
4425.5	22590	22584	(7, 8) E-x
4428.4	22575	22568	(8, 9) E-x
4431.1	22561	22557	(9, 10) E-x
4477.1	22330	22326	(10, 11) D-x
4600.6	21730	21730	(0, 3) D-x

TABLE II

State	Te ( $\text{cm}^{-1}$ )	$\omega_e$ ( $\text{cm}^{-1}$ )	$\omega_e$ Xe ( $\text{cm}^{-1}$ )
E	23077.0	405.0	1.7
D	22972.0	395.4	1.8
C	20634.0	399.0	1.5
B	22487.0	401.0	1.6
A	19001.0	409.0	1.7
X	0.0	416.0	1.4

two acids (yield: 2.5 g). Syringic acid was identified readily by the following: m.p. and m.m.; p. with an authentic sample<sup>11</sup> 203–4°; I.R.  $\nu_{\text{max}}^{\text{Nul}}$  3490  $\text{cm}^{-1}$  (OH); 2830  $\text{cm}^{-1}$  ( $\text{OCH}_3$ ); 1700  $\text{cm}^{-1}$  (COOH) and 1600  $\text{cm}^{-1}$  (Aromatic). 4-Acetyl derivative m.p. 190°; Methyl-tri-*o*-methyl, gallate, m.p. 82.5° IR  $\nu_{\text{max}}^{\text{Nul}}$  2830  $\text{cm}^{-1}$  ( $\text{OCH}_3$ ); 1730  $\text{cm}^{-1}$  ( $\text{COOCH}_3$ ) and 1600  $\text{cm}^{-1}$  (Aromatic).

The reddish-brown ether insoluble fraction (15 g) was hydrolysed with 5% methanolic hydrochloric acid followed by extraction with ether and ethyl-acetate respectively. The ether extract gave exclusively syringic acid (yield 4 g) while ethyl acetate portion (2 g) responded with a positive ferric chloride colouration for phenolics [ $R_f = 0.95$  (Brown), 0.85 (blue) and 0.72 (gray); Forestal solvent:  $\text{ACOH-Conc. HCl-H}_2\text{O}$  (30:3:10); Whatman No. 1 filter-paper; spray reagent Ferric chloride solution]. The phenolics were separated by lead acetate, and after the usual process, the mixture was crystallised from methanol as needless:  $\text{C}_{15}\text{H}_{14}\text{O}_7$ ; (Found C, 58.58; H, 4.69;  $\text{C}_{15}\text{H}_{14}\text{O}_7$  requires C, 58.82, H, 4.58%) yield. 80 mg; m.p. 188° ( $\alpha$ )<sub>D</sub> + 15.8 (C, 1.0 in  $\text{Me}_2\text{CO-H}_2\text{O}$  (1:1), I.R.  $\nu_{\text{max}}^{\text{Nul}}$  3480  $\text{cm}^{-1}$  (OH) (Broad); 3300  $\text{cm}^{-1}$  (OH), and 1600  $\text{cm}^{-1}$  (Aromatic); (blue spot,  $R_f$ : 0.85). From its reactions (blue ferric reaction and a deep red colour on heating with vanillin-HCl), this compound appears to be identical with (+) gallo-catechin<sup>12,13</sup>. It furnished a pentamethyl ether; m.p. 162° (lit. m.p. 160–2°) and a hexaacetate, m.p. 141–2° (lit. m.p. 141–3°). Direct comparison with an authentic sample could not be made.

The mother liquor from (+) gallo-catechin did not give any crystalline compound upon concentration. Further work is under progress.

The (+) gallo-catechin is a rare catechin isolated previously from a few plants like *fresh oak and chestnut bark*<sup>13</sup>, *black-wattle leaves*<sup>14</sup>, *green tea leaves*<sup>15</sup> and roots of *Rheum maximoviczii*<sup>16</sup>, etc. This is the first report of isolation of both (+) gallo-catechin and syringic acid (1% yield) from CNSL free shells of *Anacardium occidentale* Linn.

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## THERMOLYSED POLYCHELATE

THERMOLYSED polymers having high conductivity ( $\sigma$ ) exhibit a relationship between  $E_a$  (activation energy of conductivity) and  $\log \sigma_0$ , which is reverse of the compensation effect<sup>1,2</sup>. In continuation of our studies on the compensation effect observed for the polychelates of the organic ligands<sup>3</sup>, we investigated the influence of thermolysis of the polychelate of 2, 5-dihydroxy *p*-benzoquinone with copper (II). The polychelate was prepared by the method of Kanda and Saito<sup>4</sup>. The product was thermolysed at (i) 150° C. (ii) 350° C and (iii) 550° C respectively in vacuo. The electrical conductivity of the product was measured in the pellet form over a range of temperature and from these results the values of  $E_a$  and  $\log \sigma_0$  were derived (Table I).

TABLE I

Polychelate thermolysed at (°C)		$E_a$ (ev)	$\log \sigma_0$
(i)	150	0.70	2.68
(ii)	350	0.49	1.37
(iii)	550	0.30	1.70

Plot of  $E_a$  vs  $\log \sigma_0$  gave us a straight line having a slope of  $-0.38$ . Hence the thermolysed polychelate exhibits a behaviour similar to the thermolysed polymers regarding the compensation effect.

Further, the plot of  $\log \sigma$  ( $\sigma$  at  $40^\circ\text{C}$ ) vs  $1000/\text{temperature of pyrolysis}$  gives us a straight line, indicating that the conductivity of the thermolysed polychelate is a thermally activated function of pyrolysis temperature.  $E_a$  for this process is derived as  $2.48\text{ eV}$ , which is smaller than that of the thermolysed polyacrylonitrile<sup>5</sup>.

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# SYNTHESIS OF A PROTECTED NONAPEPTIDE SEQUENCE OF THE ACTIVE SITE OF TRIOSEPHOSPHATE ISOMERASE

THE enzyme triosephosphate isomerase (TPI) catalyses the interconversion, D-glyceraldehyde 3-phosphate  $\rightleftharpoons$  dihydroxyacetone phosphate, which is an important step in the metabolism of glucose<sup>1</sup>. The pentadecapeptide sequence, Trp-Val-Leu-Ala-

1 2 3 4  
Tyr-Glu-Pro-Val-Trp-Ala-Ile-Gly-Thr-Gly-Lys\*, has  
5 6 7 8 9 10 11 12 13 14 15

been shown to be part of the active site of rabbit muscle TPI<sup>1,2</sup>. The synthesis of the hexapeptide, Ala-Tyr-Glu-Pro-Val-Trp (sequence 4-9), by the solid phase method has been reported by us recently<sup>3</sup>. In continuation of our efforts to synthesise this pentadecapeptide, we now wish to report the synthesis of the protected nonapeptide sequence, Boc-Trp-Val-Leu-Ala-Tyr-Glu-(OBzl)-Pro-Val-Trp-OMe, by the classical methods.

The condensation of Boc-Val<sup>4</sup> with Leu-OMe<sup>5</sup> by the mixed anhydride<sup>6</sup> (MA) method led to Boc-Val-Leu-OMe in 80% yield, m.p.  $134^\circ$ , (Found: C, 59.16; H, 9.36; N, 8.20%.  $\text{C}_{17}\text{H}_{32}\text{N}_2\text{O}_5$  requires C, 59.28; H, 9.36; N, 8.13%). After removal of the Boc group by treatment with 3 N HCl-THF the product was coupled with Boc-Trp<sup>4</sup> by the MA method to yield Boc-Trp-Val-Leu-OMe, yield 85%, m.p.  $170^\circ$ , (Found: C, 63.65; H, 7.93; N, 11.08%.  $\text{C}_{28}\text{H}_{42}\text{N}_4\text{O}_6$  requires C, 63.37; H, 7.98; N, 10.56%). This was saponified with NaOH to furnish Boc-Trp-Val-Leu (I) in 90% yield, m.p.  $123-5^\circ$ , (Found: C, 62.44; H, 7.48; N, 11.22%.  $\text{C}_{27}\text{H}_{40}\text{N}_4\text{O}_6$  requires C, 62.75; H, 7.81; N, 10.85%).

The hexapeptide sequence, Boc-Ala-Tyr-Glu(OBzl)-Pro-Val-Trp-OMe, was obtained as follows: Dicyclohexylcarbodiimide<sup>7</sup> (DCC) mediated coupling of Boc-Val<sup>4</sup> with Trp-OMe<sup>8</sup> gave Boc-Val-Trp-OMe, yield 91%, m.p.  $149^\circ$ , (Found: C, 63.30; H, 7.47; N, 10.21%.  $\text{C}_{22}\text{H}_{31}\text{N}_3\text{O}_5$  requires C, 63.29; H, 7.48; N, 10.06%). This compound was also prepared by the MA method in 85% yield. After removal of the Boc group by treatment with 2 N HCl-EtOAc in presence of mercaptoethanol, the product was reacted with Boc-Pro<sup>4</sup> by both DCC and MA methods to yield Boc-Pro-Val-Trp-OMe, in 88% and 91% yields respectively, m.p.  $98-100^\circ$ , (Found: C, 63.01; H, 7.57; N, 10.48%.  $\text{C}_{27}\text{H}_{38}\text{N}_4\text{O}_6$  requires C, 63.02; H, 7.44; N, 10.89%). Similar deprotections and DCC catalysed condensations were carried out for the introduction of Boc-Glu(OBzl)<sup>9</sup>, Boc-Tyr<sup>4</sup> and Boc-Ala<sup>4</sup> to yield Boc-Glu-(OBzl)-Pro-Val-Trp-OMe, yield 86%, m.p.  $106-8^\circ$ , (Found: C, 63.78; H, 7.00; N, 9.88%.  $\text{C}_{39}\text{H}_{51}\text{N}_5\text{O}_9$  requires C, 63.83; H, 7.02; N, 9.55%), Boc-Tyr-Glu(OBzl)-Pro-Val-Trp-OMe, yield 89% m.p.  $112-5^\circ$ , (Found: C, 64.46; H, 6.80; N, 9.43%.  $\text{C}_{48}\text{H}_{60}\text{N}_6\text{O}_{11}$  requires C, 64.27; H, 6.74; N, 9.37%) and Boc-Ala-Tyr-Glu(OBzl)-Pro-Val-Trp-OMe (II), yield 88%, m.p.  $124-7^\circ$ , (Found: C, 63.10; H, 6.70; N, 10.27%.  $\text{C}_{51}\text{H}_{65}\text{N}_7\text{O}_{12}$  requires C, 63.27; H, 6.76; N, 10.13%).

The Boc group in the hexapeptide (II) was removed and the product was acylated with the mixed anhydride prepared from the tripeptide (I) to yield the nonapeptide, Boc-Trp-Val-Leu-Ala-Tyr-Glu(OBzl)-Pro-Val-Trp-OMe in 66% yield, m.p.  $171-3^\circ$ , (Found: C, 64.03; H, 6.98; N, 10.91%.  $\text{C}_{73}\text{H}_{95}\text{N}_{11}\text{O}_{15}$  requires C, 64.19; H, 6.95; N, 11.28%), corresponding to the sequence 1-9 of the pentadecapeptide.

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\* All the amino acids (except glycine) have the L configuration. Abbreviations conform to the recommendations of IUPAC-IUB Commission on Biochemical Nomenclature.

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#### SPECTRAL STUDIES OF THE RARE EARTH CHELATES OF TRIDENTATE SCHIFF BASES

N-SALICYLIDENEANTHRANILIC acid ( $H_2SA$ ) and N-salicylidene- $\beta$ -alanine ( $H_2S\beta A$ ) form solid chelates with La(III), Ce(III), Pr(III), Nd(III) and Sm(III). The magnetic susceptibility and infrared spectra of these chelates have been discussed in this communication.

*Synthesis of  $H_2SA$  and  $H_2S\beta A$ .*—Freshly distilled salicylaldehyde (1.2 g) in ethanol was added to ethanolic solution of anthranilic acid (1.2 g) or aqueous-ethanol solution of  $\beta$ -alanine (0.89 g) and the mixture was refluxed on a water-bath for an hour when an orange yellow mass of the Schiff base was formed. After the reflux, on cooling beautiful orange yellow crystals of  $H_2SA$  or  $H_2S\beta A$  separated out. These were recrystallised from absolute alcohol. The yield was found quantitative.

*Analysis.*—Found, C, 69.52, H, 4.4 and N, 5.6, calcd. for  $C_{14}H_{11}NO_3$ , C, 69.70, H, 4.56 and N, 5.80% m.p. 201°. Found, C, 62.05, H, 5.4 and N, 7.10. Calcd. for  $C_{10}H_{11}NO_3$ , C, 62.17, H, 5.6 and N, 7.25% m.p. 126°. Their molecular weights were found to be 241 ( $H_2SA$ ) and 193 ( $H_2S\beta A$ ) as determined ebullioscopically by Gallenkamp Semi-micro ebulliometer. These Schiff bases contain a carboxylic, phenolic, hydroxyl and an imino group and are thus biprotic tridentates. With these Schiff bases Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) form chelates which have already been reported<sup>1</sup>.

*Synthesis of Rare Earth Chelates.*—To an ethanolic-solution of  $H_2SA$  or  $H_2S\beta A$  (0.023 M), a solution of rare earth metal nitrate (0.02 M) in 80% ethanol was added gradually and the mixture was stirred magnetically. Dilute ammonia (1:20) was then added dropwise to this mixture until a flocculent mass was obtained which was stirred continuously for 4–5 hrs. The mass was filtered under suction, washed with hot ethanol, dried and preserved in a vacuum desiccator. The yield and their analysis are summarised in Table I. These chelates possess 1:2 metal-ligand stoichiometry.

TABLE I

Analyses, yields and the  $\mu_{eff}$  values of rare earth metal chelates of  $H_2SA$  and  $H_2S\beta A$

Composition of chelate	Yield %	Analyses				$\mu_{eff}$ B.M. (at 303°K)
		Found %		Calculated %		
		N	Metal	N	Metal	
H <sub>2</sub> SA—Chelates						
LaC <sub>28</sub> H <sub>19</sub> NO <sub>6</sub>	85	4.28	22.07	4.53	22.47	..
CeC <sub>28</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	74	4.31	22.39	4.48	22.48	2.21
PrC <sub>28</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	71	4.43	22.58	4.51	22.74	3.31
NdC <sub>28</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	78	4.18	22.90	4.49	23.14	3.59
SmC <sub>28</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	78	4.21	23.64	4.44	23.88	1.53
H <sub>2</sub> SβA—Chelates						
LaC <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	80	5.21	26.49	5.36	26.63	..
CeC <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	75	5.34	26.81	5.53	26.94	2.19
PrC <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	72	5.22	26.73	5.34	26.89	3.33
NdC <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	80	5.21	27.15	5.31	27.35	3.57
SmC <sub>20</sub> H <sub>19</sub> N <sub>2</sub> O <sub>6</sub>	78	5.17	27.97	5.24	28.21	1.58

*Ligand Replacement Reactions.*—Bis(N-salicylideneanthranilato) or Bis(N-salicylidene- $\beta$ -alaninato) metal chelate (0.01 M) thus prepared, was added to a suspension of EDTA (0.01 M) in water and the mixture was heated on a boiling water-bath for nearly two hrs. On cooling,  $H_2SA$  or  $H_2S\beta A$  separated out which was extracted into chloroform. The aqueous solution on concentration and cooling gave the crystals of EDTA-chelate of the rare earth. These were dried and preserved in a vacuum desiccator.

The chloroform extract was concentrated and cooled when  $H_2SA$  or  $H_2S\beta A$  crystals separated out. The yield of the Schiff base recovered was

found almost quantitative in all cases, which indicate a complete exchange of ligands.

The magnetic susceptibility determinations and infrared spectral studies of these rare earth chelates were carried out on a Gouy apparatus and a Perkin Elmer Spectrophotometer in Nujol mull respectively.

**Results and Discussion.**—An examination of the Infrared Spectra of the Schiff bases has shown that there are two bands at  $3440\text{ cm}^{-1}$  and  $2570\text{ cm}^{-1}$  which can be assigned to the presence of phenolic  $-\text{OH}$  and carboxylic  $-\text{OH}$  respectively. In all the metal chelates under study the weak band at  $2570\text{ cm}^{-1}$  could not be located which suggests its elimination due to complexation. But in the spectra of the metal chelates (having 1:2 metal-ligand stoichiometry, Table I) the presence of only one band at  $3050\text{ cm}^{-1}$  indicates the presence of a bonded phenolic  $-\text{OH}$ . This  $-\text{OH}$  appears to be coordinated to the central metal atom so as to complete its coordination number six.

The Schiff bases have also shown two more bands at  $1670\text{ cm}^{-1}$  and  $1690\text{ cm}^{-1}$  which suggest the presence of  $\text{C}=\text{N}$  and  $\text{C}=\text{O}$  stretching vibrations respectively. In the spectra of metal chelates these bands are broadened and no marked shift in the carboxyl frequency was observed. It has been reported that in the spectra of the rare earth chelates of Salicylaldehyde<sup>2</sup> and Acetoacetanilide<sup>3</sup> shift in the carbonyl frequency was also not observed. The weak band at  $2750\text{ cm}^{-1}$  due to aldehydic  $\text{C}-\text{H}$  vibrations could neither be located in the spectra of the Schiff bases nor in those of the metal chelates which might be due to the merger of  $\text{C}-\text{H}$  vibrations in the Nujol absorptions in this region.

The La(III) chelate under examination was found to be diamagnetic. The magnetic moments (Spin-free values, Table I) obtained experimentally for the remaining rare earth chelates are in good agreement with the values for typical Lanthanide Sulphates<sup>4</sup>. These values suggest that the Lanthanide ion acts, approximately as free ion, as far as the  $f$ -electrons are concerned.

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#### A RAPID METHOD FOR CLASSIFYING GRANITIC ROCKS FROM X-RAY FLUORESCENCE INTENSITIES OF ROCK POWDERS

GRANITIC rocks are important repositories of numerous economic minerals, and are potentially profitable resources of many strategic rare metals<sup>1</sup>. Of the various factors that dictate, whether or not, a granitic body is likely to contain a certain rare metal in sufficient concentration to be commercially exploitable, petrography is a very important one. Precise classification and nomenclature of granitic rocks, therefore, are of utmost importance in mineral prospecting. As large areas of our country are composed of granitic rocks, and our knowledge regarding their petrography is inadequate, it is essential to subdivide each granitic terrain into units of tonalite, granodiorite, adamellite, and granite. Traditional methods of petrographically naming granitic rocks involve preparation of transparent thin sections, differential staining of constituent minerals of thin sections using reagents like HF, and modal analyses of stained thin sections by microscopic point-counting, or by linear integration. Such methods are time-consuming, laborious, and tedious. The purpose of this note, therefore, is to propose a very rapid and accurate method for precisely classifying and naming granitic rocks.

**Procedure.**—(1) Representative portions of granitic rock samples are ground to about 400 mesh using high-speed impact and swing mills, e.g., shatterbox, mixer mill, nylon sieves. (2) The finely ground rock powders are pelletized by pressing them against stainless steel discs, with a suitable backing like borax or bakelite, in a die at about 30,000 psi pressure using a soil test press. (3) The  $\text{K}_2\text{O}$  and  $\text{CaO}$  contents of each pellet are estimated by standard practices of X-ray fluorescence techniques. (4) Each granitic rock sample is then precisely classified and named on the basis of its  $\text{K}_2\text{O}/\text{CaO}$  ratio (tonalite if  $< 0.75$ ; granodiorite if  $0.75$  to  $1.5$ ; adamellite if  $1.5$  to  $3$ ; granite if  $> 3$ ).

**Evaluation of the Method.**—Ten granitic rock samples from the 2,700 million year old Giants Range batholith of northeastern Minnesota<sup>2</sup>, whose petrographic names were obtained from traditional modal analysis (Fig. 1, top), were pelletized. The pellets were irradiated in a Norelco X-ray fluorescence unit equipped with a tungsten tube, EDDT analyzing crystal, and flow proportional counter. The intensities of  $\text{KK}_\alpha$  and  $\text{CaK}_\alpha$  characteristic

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radiations were converted to oxide compositions using calibration curves constructed from the  $KK_{\alpha}$  and  $CaK_{\alpha}$  intensities obtained for pellets of the U.S.G.S. rock standards<sup>3,4</sup> G-1, G-2 and GSP-1.

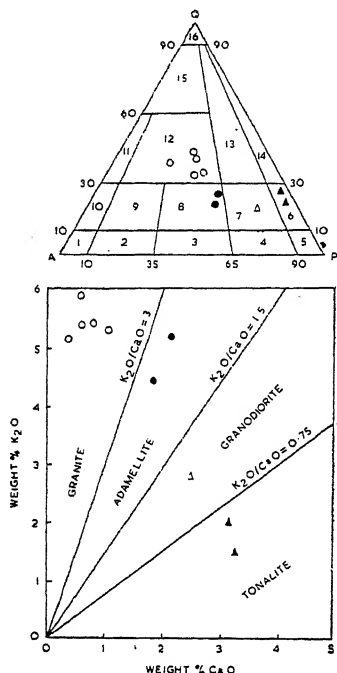


FIG. 1. (Top) Modal Quartz(Q)-Alkali feldspar(A)-Plagioclase(P) ternary diagram for classification of granitic and related rocks<sup>1</sup>; 1. Alksyenite; 2. Syenite; 3. Monzonite; 4. Monzodiorite; 5. Diorite; 6. Tonalite; 7. Granodiorite; 8. Adamellite; 9. Syenogranite; 10. Alksyenogranite; 11. Alkgranite; 12. Granite; 13. Quagranodiorite; 14. Quatonalite; 15. Quagranite; 16. Quartz vein. (Bottom)  $K_2O$ - $CaO$  binary diagram for classification of granitic rocks.

The  $K_2O$  and  $CaO$  contents so obtained were plotted on a  $K_2O$ - $CaO$  binary diagram. The petrographic names of the ten granitic samples derived from the diagram (Fig. 1, bottom) are identical with those obtained from modal data (Fig. 1, top)—two tonalites, one granodiorite, two adamellites, and five granites—thus confirming the correctness of the method.

**Conclusion.**—The speed and accuracy of the X-ray fluorescent method proposed herein are such that one operator, with an assistant, can correctly obtain the precise petrographic names of as many as 500 granitic rock samples in an eight-hour working day, as against a mere 5–10 samples by the traditional methods of microscopic petrography. Such speed and accuracy are absolutely essential for facies characterization of Indian granitic terrains

into units of tonalite, granodiorite, adamellite, and granite.

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#### BLIGHT DISEASE OF SUNFLOWER CAUSED BY *ALTERNARIA HELIANTHI* (HANSF.) TUBAKI AND NISHIHARA IN INDIA

A SEVERE blight disease of sunflower (*Helianthus annuus* L.) grown for the purpose of germ plasm (Exotic collection) maintenance was observed during September 1973, in the fields of Plant Introduction Division of the Indian Agricultural Research Institute, New Delhi. All the above ground plant parts, i.e., stem, leaves, petioles and flowers were severely affected. In advanced stage of the disease, plants showed heavy defoliation.

Isolations on Potato Dextrose Agar (PDA) from young necrotic spots revealed the association of a species of *Alternaria*. On PDA the fungus grew rather slowly but with abundant sporulation.

Conidiophores are cylindrical, isolated or in groups, pale grey to pale yellow, straight or curved, geniculate, simple or branched, upto 7 septate and measure  $38.40\text{--}138.24 \times 5.76\text{--}11.52 \mu$  (Fig. 1). The conidia are cylindrical to long ellipsoid, mostly straight or slightly curved, light yellow to yellowish brown with rounded ends. The number of transverse septa varies from 1–11. (Av. 6 septa) while 1–3 longitudinal septa are frequently present. The conidia are prominently constricted at transverse septa and measure  $23.04\text{--}138.24 \times 15.36 \times 38.40 \mu$  (host); and  $53.76\text{--}149.76 \times 13.44\text{--}34.56 \mu$  (culture) (Fig. 2). On the basis of above characters the fungus was identified as *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. However, in the present isolate chains of conidia (2–3 in a chain), were commonly observed (Fig. 3) both in culture as well as on the diseased host bits incubated in moist chambers, but Tubaki and Nishihara<sup>2</sup> state that

'there is no evidence that chains of conidia are produced' in their isolate.

For determining the pathogenicity of the fungus ten-day old monospore culture maintained on PDA was used. One-month old sunflower plants were sprayed with conidial suspension in water.

First symptoms were observed 24 hours after inoculation on the leaves in the form of numerous light brown to brown flecks and oval to circular spots surrounded by chlorotic halo, measuring 1-2 mm in diameter. Such spots gradually increased in size and coalesced to form bigger irregular or circular spots, 2-3 cm in diameter. Severely infected leaves showed inward curling of the margins resulting in complete blighting and drying up. On petioles, flowers and stems, infection appeared in the form of light brown elongated spots or streaks of variable size. Cracking of stems (Fig. 4) and petioles was very prominent. Within

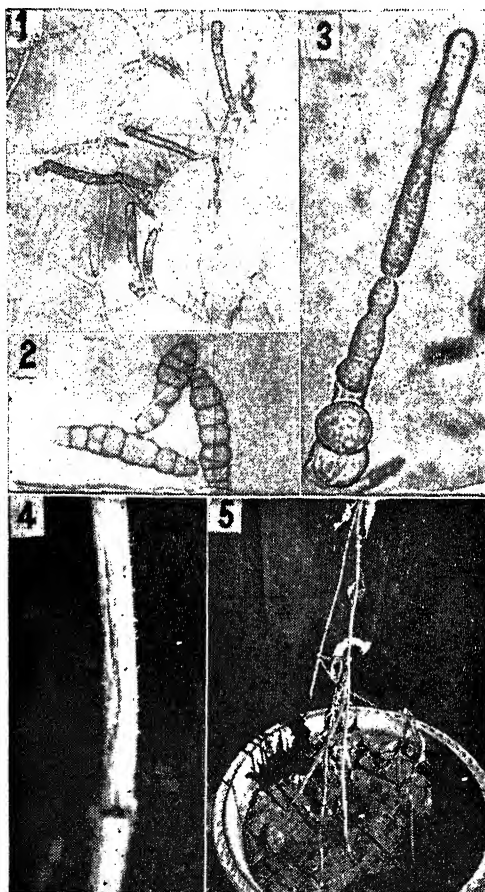
10-15 days all the inoculated plants were completely blighted (Fig. 5).

Reisolations from all the infected tissues on PDA repeatedly yielded *A. helianthi*. In preliminary tests the fungus failed to infect chrysanthemum (*Chrysanthemum indicum* L.) plants. *A. helianthi* and *A. chrysanthemi* according to the previous reports<sup>1-5</sup>, do not develop conidia in chains. Ellis (per. com.) has stated that the two species are morphologically indistinguishable. The only difference is that the isolates from *Helianthus* are unable to infect *Chrysanthemum* and vice versa. Accordingly, inoculation were made on *Chrysanthemum indicum* but no infection developed. Although in the recent report of Anil Kumar *et al.*<sup>1</sup>, the fungus recorded on sunflower in Bangalore has been identified as *A. helianthi*, it may be noted that they have not carried-out cross inoculation studies nor they have observed development of conidia in chains. A comparative study alone would be able to throw light on these aspects.

The culture of the fungus has been deposited with "Indian Type Culture Collection" at the Division of Mycology and Plant Pathology, I.A.R.I., New Delhi (Acc. No. 1828) and Commonwealth Mycological Institute, Kew, Surrey, England (IMI. 191102).

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FIGS. 1-5. Fig. 1. Conidiophores,  $\times 158$ . Fig. 2. Conidia,  $\times 158$ . Fig. 3. Conidia in chain,  $\times 382$ . Fig. 4. Cracking of the stem due to infection. Fig. 5. Plants showing blight symptoms.

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STUDIES ON THE TOXINS OF *PYRICULARIA*

## 2. Detection of Phiriculol in Blast Diseased Leaves of Gramineae

TAMARI AND KAJI considered  $\alpha$ -picolinic acid and piricularin as the toxins of *Pyricularia oryzae* and detected both compounds in extracts from diseased rice plants<sup>7</sup>. However, all attempts in this laboratory to identify these two toxins in cultures of *Pyricularia* from rice or other Gramineae and in diseased leaves of their respective hosts have not been successful. Recently, other phytotoxic substances, viz., pyriculol<sup>2</sup>, 3, 4-dihydro-3, 4, 8-trihydroxy-1(2H)-naphthalenone<sup>1</sup> and tenuazonic acid<sup>1</sup> have been isolated from cultures of the rice blast fungus. Of these, tenuazonic acid has also been isolated from blast-diseased rice plants<sup>8</sup>. Although pyriculol has been shown to cause a dark necrotic spot, resembling the natural blast lesion, on rice leaves and also inhibit growth of rice seedlings<sup>5,6</sup>, this compound does not appear to have been detected in blast-diseased leaves of rice or other gramineous hosts. It has been reported that pyriculol could be detected not only in cultures of *P. oryzae* but also in cultures of *Pyricularia* from other cultivated and wild Gramineae<sup>4</sup>. It was, therefore, of interest to study if pyriculol could be detected in blast-diseased leaves of various Gramineae.

Six gramineous hosts, viz., *Oryza saliva* (CO 13), *Setaria italica* (CO 1), *Eleusine coracana* (CO 5), *Panicum repens*, *Brachiaria mutica* and *Leersia hexandra* were raised in a temperature controlled growth room<sup>3</sup>. The six hosts were inoculated with conidial suspensions of the respective isolates of *Pyricularia*. Lesions were collected on the fourth day of inoculation when the symptoms were discernible by their water-soaked appearance.

One gram each of the lesions was extracted with 80% methanol in a mortar using acid washed sand. The extracts were centrifuged at 7,000 r.p.m. for 15 min and the methanol in the supernatant driven off *in vacuo* at 50° C. The aqueous residue was reprecipitated and the supernatant extracted with 3 volumes of ethyl acetate. The ethyl acetate extracts were pooled and taken to dryness *in vacuo* at 50° C. The residue was dissolved in 1.0 ml of ethyl acetate and 0.5 ml of the sample was streaked on a 250  $\mu$  thick silica gel (without binder) thin-layer plate previously activated at 120° C for 30 min. Authentic pyriculol was used as the marker. The plate was first developed with benzene and subsequently with benzene : ethyl acetate (9 : 1, 8 : 2 and 7 : 3 v/v) solvent systems to a distance of 100 mm. Authentic pyriculol could be located at an  $R_f$  of 0.39 under U.V. (356 nm) after the final development. The marker and the region cor-

responding to the marker were scrapped off, eluted with ethanol and scanned in the U.V. region for their absorption spectra in a UNICAM SP 800 Spectrophotometer. The spectra of the samples were compared with that of authentic pyriculol.

Of the extracts from the six gramineous hosts studied, pyriculol was detectable only in the case of blast lesions from *B. mutica* (Fig. 1) although

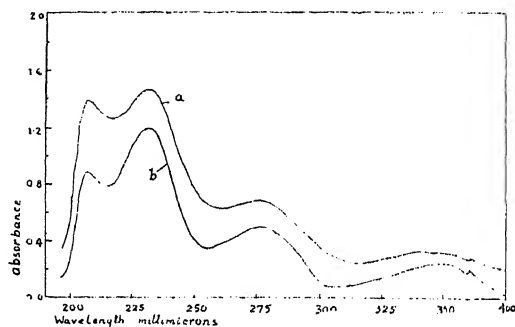


FIG. 1. U.V. spectra of pyriculol detected in blast lesions from *B. mutica* (a) and authentic pyriculol (b) in ethanol.

the compound was detectable in culture filtrates of all the isolates used here except the one from rice<sup>4</sup>. The amount of pyriculol in blast lesions from *B. mutica* was estimated from the O.D. value at 232 nm with reference to known standards prepared from authentic pyriculol and this amounted to 132  $\mu$ g/g fresh weight of lesions. Although pyriculol could not be detected in blast lesions from the other hosts, its detection *in vivo* reported here and that it can be frequently isolated from cultures of *Pyricularia* from many gramineous hosts<sup>4</sup> suggest a possible role for this polyketide in the blast disease syndrome.

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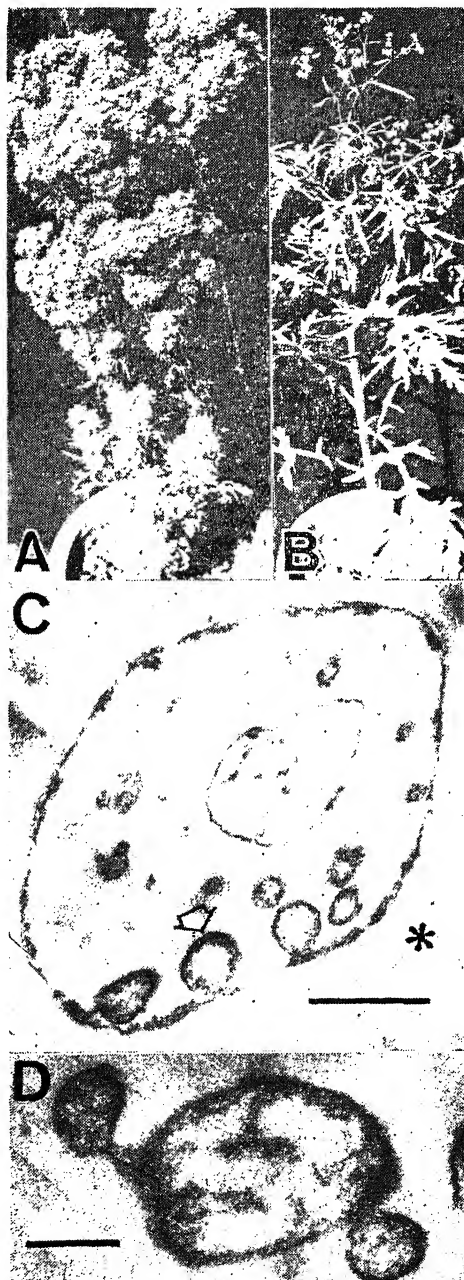
The authors are thankful to Dr. Pritthipal Singh, Deshbandhu Gupta College, New Delhi, for identification of the plant.

### PROBABLE MYCOPLASMAL ETIOLOGY OF BROOM BUSH WITCHES' BROOM

BROOM-BUSH (*Parthenium hysterophorus* Linn., Fig. B), a recent introduction in the country, has widely spread in the plains and in the hills<sup>1</sup>. The plants can be used for some medicinal purposes<sup>2</sup> but at present it is a ravaging weed. In 1972-73 typical witches' broom symptoms (Fig. A) were noticed around Delhi in a large number of affected plants of *P. hysterophorus*. Such plants branched profusely, each branch remained feeble and thin, the size of the leaves and internodes considerably reduced, and produced minute phylloid flowers. The plants become solid masses of small leaves looking like large green sponges from a distance. The symptoms are typical of yellows type of diseases now associated with mycoplasma-like organisms. Therefore, ultrathin sections of phloem tissues from diseased and healthy plants of *P. hysterophorus* were examined in an electron microscope and the results are reported here.

For electron microscopy, small pieces of stem and leaves were fixed in 2% glutaraldehyde in cold for 12 hrs followed by postfixation in 1% osmium tetroxide in 0.05 M cacodylate buffer pH 7.2 for 2 hrs. The tissues were dehydrated in acetone and embedded in Spurr's low-viscosity embedding medium. Ultrathin sections, cut with LKB ultratome, were post stained with uranyl acetate and lead citrate and examined in Philips EM 300.

Sieve tubes of phloem tissue contained typical mycoplasma-like bodies (Figs. C and D), whereas, no such bodies were found in corresponding healthy tissues. Each MLB was limited by a membrane and contained fibrous nuclear material and granular ribosome-like structures. Some of the bodies were found budding indicating a possible mode of multiplication of these bodies. The size of the MLBs varied from 126 to 850 nm. Filamentous forms were not observed. As MLBs were not found in healthy tissues, the disease is probably caused by mycoplasma-like organism. Whether the disease can be used for biological control of this obnoxious weed remains to be seen.



FIGS. A-D. A. diseased plant of broom-bush in a pot. B. A healthy broom-bush. C. Mycoplasma-like bodies (arrow) in a sieve tube of diseased stem tissue. Asterisk indicates cell wall and the bar represents 500 nm. D. MLBs in budding stage, bar represents 250 nm.

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### WILD RICE PLANTS AS POSSIBLE SOURCE OF BACTERIAL BLIGHT INOCULUM OF CULTIVATED RICE

XANTHOMONADS in general are very specific in their parasitism, and comparatively narrow in their host-range. However, several collateral hosts have been reported for *Xanthomonas oryzae* in Japan<sup>7-9,16-18</sup>, Korea<sup>12</sup>, Philippines<sup>3</sup> and India<sup>2,13</sup>. Out of thirteen weed hosts reported only *Leersia sayanuka*, *L. oryzoides* and *Zizania latifolia* were found to be the natural hosts of the pathogen in Japan and Korea whereas on the other ten species no natural infection could be observed. Devadath reported that the pathogen was found to survive in wild rice, *Oryza perennis* Moench. in Orissa<sup>4</sup>. This was further confirmed by other workers in India<sup>10</sup> and Australia<sup>1</sup>.

The ability of the casual bacterium to infect plants other than rice poses a serious problem in the formulation of effective control measure. No comprehensive study has yet been made on the host range of *X. oryzae* in India. Therefore, fifteen species of Gramineae, viz., *Andropogon annulatus* Forsk., *Brachiaria mutica* Stapf., *Digitaria sanguinalis* (L.) Scop., *Digitaria setigera* Roth ex Roem et Schult., *Echinochloa colonum* (L.) Lk., *Eleusine aegyptiaca* Desf., *E. indica* (L.) Gaertn., *Eragrostis coarctata* Stapf., *Imperata arundinacea* Cyrill., *Ischaemum ciliare* Retz., *Leersia hexandra* Swartz., *Panicum indicum* L. var. *gracile*, *Panicum muticum* Forsk., *Panicum proliferum* Lam., *Setaria glauca* Beauv., four species of Cyperaceae, viz., *Cyperus inundatus* Roxb., *Fimbristylis ferruginea* Vahl., *F. milicaca* Vahl., *F. thomsonii* Boeck., twenty-two wild rice species, viz., *Oryza perennis* Moench., *O. grandiglumis* (Doell) Prod., *O. punctata* Kotschy ex Steud., *O. brachyantha* A. Cheval. et Roerich., *O. barthii* A. Cheval., *O. australiensis* Domin., *O. officinalis* Wall ex Watt., *O. perrieri* A. Camus., *O. latifolia* Desv., *O. schweinfurthiana* Roschev., *O. subuiata* Nees, *O. rufipogon* Griffiths., *O. eichingeri* A. Peter., *O. jeyporensis* S. Govindaswami., *O. longiglumis* Jansen., *O. malampuzhensis*

Krish et Chand., *O. ridleyi* Hook f., *O. alia* Swallen., *O. coarctata* Roxb., *O. sativa* L. v. *fatua* Prain., *O. granulata* Nees et Arn. ex Hook f., and *O. minuta* J.S. Presl ex C.B. Presl., and eight cereals and millets, viz., *Triticum vulgare* Vill., *Eleusine coracana* (L.) Gaertn., *Setaria italica* (L.) Beauv., *Pennisetum typhoides* (Burm.) Stapf. and Hubbard. *Sorghum vulgare* Pers., *Zea mays* L., *Paspalum scrobiculatum* L. and *Panicum miliaceum* L. were tested against a virulent isolate of *X. oryzae*. The plants were grown in pots and manured with 40 lb of nitrogen.

The test species were inoculated by spraying with 24 hr old bacterial suspension as per Devadath and Padmanabhan<sup>5</sup>. Inoculated plants were kept in moist chambers for 48 hr and replaced in green house and observed for disease development upto 21 days. If no symptoms developed upto 21 days they were reinoculated and observed again.

Amongst the 49 species listed only the twenty-two wild rices were found to be susceptible. Multiple pin prick inoculation was avoided in the study because the pin pricks themselves may cause reddish restricted lesions and the bacterium may colonize in this tissue.

Goto<sup>6</sup> tested 18 wild rice species and concluded that they were all susceptible to the virulent isolate B 72 while some were resistant to the less virulent isolate BP 1.

Besides Goto<sup>6</sup>, Dalmacio and Exconde<sup>3</sup> have also tested some of the weeds, cereals, millets and wild rices included in the current tests and similar results were obtained. Pandey<sup>11</sup> tested 32 common Indian weeds and the pathogen established only in six of the weeds successfully, around the site of inoculation but none of them could be considered to be a possible active host.

The weeds reported as susceptible to *X. oryzae* in Japan, Philippines and Korea are not known to occur in endemic areas of India<sup>15</sup>. The susceptibility of *C. deformis* and *C. rotundus*<sup>2</sup> and *L. hexandra*<sup>13</sup> could not be confirmed by other workers<sup>3</sup>.

Wild rice species like *O. officinalis*, *O. jeyporensis*, *O. perennis*, *O. malampuzhensis*, *O. coarctata* and *O. granulata* are known to occur in different parts of India<sup>14</sup>. These were observed to be infected in ponds and irrigation canals earlier than the rice crop in the States of Karnataka, Andhra Pradesh, Orissa, Bihar, West Bengal, Manipur, Tripura, Assam and Meghalaya. The source of inoculum from wild rices has also been noted in Maharashtra<sup>10</sup>. The high degree of susceptibility of these wild rice species under artificial inoculation tests and the occurrence of infected wild rices under natural conditions tend to support the view



that they may play an important role in the ecology of the bacterium. How far these wild rices contribute to the development of bacterial blight epidemic on cultivated rice is yet to be studied.

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#### PYTHIUM MIDDLETONII PARASITIC IN THE ROOTS OF CRUCIFERS

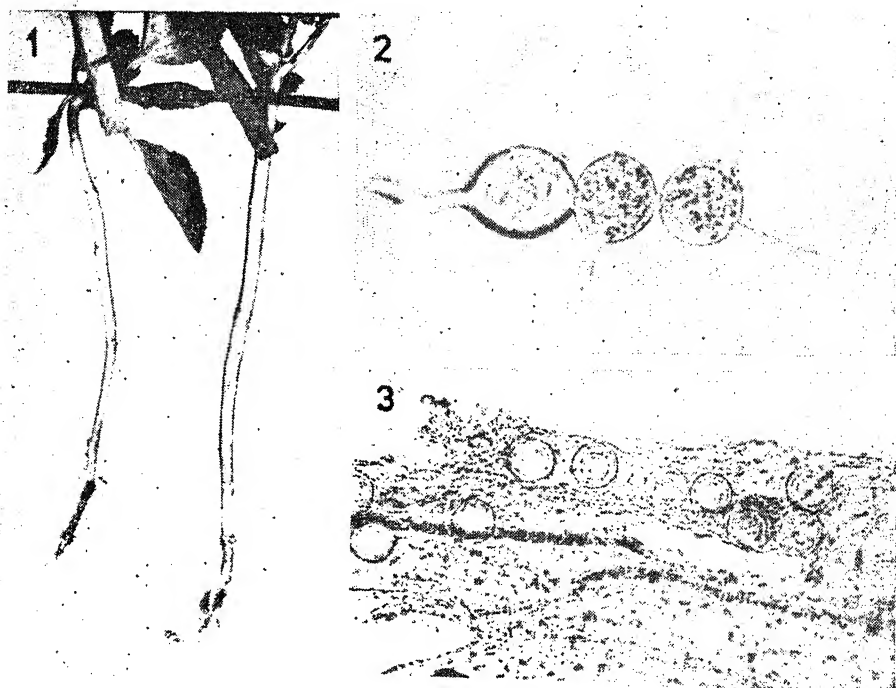
A DESTRUCTIVE root rot disease of cauliflower (*Brassica oleracea* L. var. *botrytis*) and cabbage (*Brassica oleracea* L. var. *capitata*) incited by a species of *Pythium* has been recurrently noticed especially in the low-lying, ill-drained, heavy soil fields of Varanasi, U.P. Although the disease is prevalent in early transplanted cauliflower (August to September), the pathogen is seen more destructive to cabbage during December to January soon after the winter rains. The losses due to the disease range between 12-15% and under favorable environment, may reach even 20%. The high disease

incidence has been observed associated with water-logged soils and humid weather after the rains prevalent until head formation. Both the host plants appear susceptible to infection in all ages.

Disease incidence is noticed in the field as pale yellow discoloration of the crown and curling of the maturing leaves followed by sudden wilt and death of the plant. The infection initiates near the tips of tender rootlets, which soon travels upward and the pathogen produces a soft rot in the root cortex and later in the vascular tissues involving the entire root system and part of the underground stem (mesocotyl). Sometimes only the roots become severely rotted, leaving the mesocotyl free from infection (20-30 days after transplantation). The cortex of the tap and lateral roots becomes water-soaked and softened, usually sloughs off and remains in the soil, when the plant is pulled out (40 days after transplantation). Secondary organisms make their entrance in the infection sites subsequently (Fig. 1). Infection is also observed in the nursery beds. The tender roots of 2-week old seedlings are infected, the seedlings topple over the ground and ultimately die. Seedlings bearing incipient infection and transplanted from the nursery beds soon become severely diseased in the field under the spell of favorable environment. The cortical tissues of the roots and mesocotyls in the infected seedlings/plants become loaded with oospores of the pathogen helping its survival in the soil and build-up of the primary inoculum for the succeeding crop season (Fig. 3).

The pathogen is readily isolated on corn meal agar (CMA) and potato dextrose agar (PDA) from diseased tissue, which consistently yielded a species of *Pythium* Pringsh. The mycelium is hyaline glassy, the hyphae being 5-9  $\mu$  in diam. Occasionally septa are observed in older hyphae in stalling cultures. Zoosporangia are subspherical to globose, smoothwalled, hyaline papillate, measuring 18.5-29  $\mu$  (avg. 26.5  $\mu$ ) in diam. and releasing 15-30 zoospores through an evacuation tube (Fig. 2). The zoospores are hyaline, bean-shaped, biciliate and 7-13  $\mu$  in diam. after coming to rest. Sexual organs abundantly develop on CMA. The oogonia are spherical to subspherical, smooth, thin-walled, terminal or intercalary bearing a short apical papilla, measuring 20.5-33.5  $\mu$  (avg. 25.5  $\mu$ ) in diam. Antheridia are typically monoclinal, terminal, clavate, making an apical contact with the oogonial wall and measure 5.5-8.5  $\times$  11.5-16.5  $\mu$  (avg. 7.5  $\times$  14.5  $\mu$ ). The oospores are round, thick-walled, smooth, hyaline, containing a slightly prominent reserve globule; a single oospore always completely fills the oogonium and measures 22-27.5  $\mu$  (avg. 23.5  $\mu$ ) in diam.





FIGS. 1-3. Fig. 1. Cauliflower seedlings with root rot by *Pythium middletonii*, ( $\times 1/3$ ); Fig. 2. Zoosporangia bearing exit tubes, ( $\times 500$ ); Fig. 3. Oospores in the disintegrating cortex, ( $\times 200$ ).

Inoculations with mycelial fragments as well as zoospores on the host seedlings raised in sterile sand gave positive results developing symptoms identical to those in the field 9-13 days after inoculation. Similar inoculations on the seedlings of other crucifers such as mustard (*Brassica campestris* L.), turnip (*Brassica napus* L.), knolkhol (*Brassica oleracea* L. var. *caulorapa*) and radish (*Raphanus sativus* L.) resulted in a mild infection of mustard and radish but none of the other host seedlings. Cultural characters and morphology of the pathogen under study indicate its identity with *Pythium middletonii* Sparrow, to which it is referred (IMI 173182). The species described and renamed by Sparrow<sup>1</sup> was apparently isolated as a saprophyte from water. Review of literature revealed that its parasitism has hitherto not been reported on any plant so far and its occurrence here constitutes the first record for India.

The first author (S.L.S.) expresses his gratitude to the Indian Council of Agricultural Research, New Delhi, for the award of a Senior Research Fellowship; to the Director, Commonwealth Mycological Institute, Kew, England, for kindly providing identification of the species.

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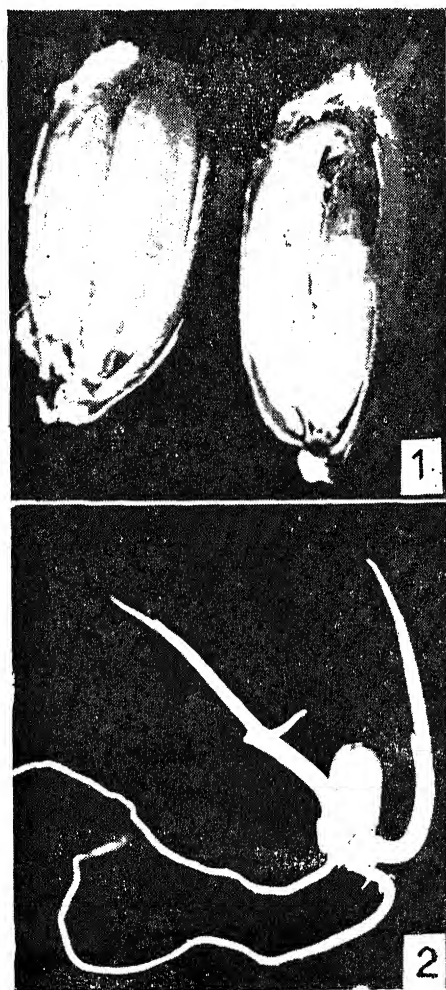
#### INDUCED TWINNING IN RICE

PRESENCE of double grains in some rice varieties has been reported in world genetic stock maintained at Central Rice Research Institute, Cuttack (Anonymous, 1971). Only four varieties, Ac. 852, Ac. 1225, Ac. 2521 and JBS. 1210 belonging to late duration group from tropical zone (23° N-23° S) were found to be double-grained. However, double-grained varieties are not noticed in temperate zone (23°-40° N and 23°-40° S) rice varieties.

In the present study one popular cultivated variety from temperate zone (Kashmir, 34° N Lat), namely, Ch. 1039 which is an introduction from China, was subjected to mutagen treatment with 1% Ethyl Methane sulphonate. One double-grained mutant was isolated from M<sub>2</sub> generation. The frequency

of occurrence of this mutant was one in 10,608  $M_2$  plants (0.009%).

In this mutant two kernels were developed from two separate ovaries formed within a single spikelet (Fig. 1). The mutant spikelet consisted of two separate carpels and six stamens. In a fully developed grain the two kernels were completely enclosed by the lemma and palea. The double-grained spikelets were slightly heavier and bolder than the normal grains. Fully developed embryos were formed in both the kernels which on germination gave two separate viable seedlings (Fig. 2).



FIGS. 1-2. Fig. 1. Two spikelets of double grain mutants showing two well-developed kernels. Fig. 2. Double grain mutants on germination producing two viable seedlings.

Similar observations were also recorded in an  $M_2$  population of gamma-ray-treated (20 Kr) seeds of a culture designated as CRHP, 8 which was

bred by crossing *japonica* and *indica* varieties (Gaisen Mochi  $\times$  Pirurutong). The frequency of occurrence of such mutant was 5 out of 4000  $M_2$  plants (0.125%).

Twin seedlings obtained from a single embryo or from double-grained seeds are often considered a good source for the production of haploids. But in this case, progenies grown out of these twin seedlings always produced only normal diploid plants and cytological examination did not reveal the presence of any numerical or structural aberrations.

One interesting feature observed in this mutant was that all the spikelets in a panicle are not double-grained. The frequency of double-grained spikelets in a panicle varied from 8.3% to 40.9% in Ch. 1039, and 1.2 to 5.8% in CRHP, 8 mutants, indicating incomplete penetrance and variable expressivity of this character. Similar findings have also been reported by Rana (1966) in induced twinning in barley.

Bhatia and Swaminathan (1963) and Raj *et al.* (1972), likewise reported the occurrence of multiple carpels in wheat and rice respectively. But in both these cases no seed setting was observed. The present report is the first instance of induced twinning in rice where two well-developed functional carpels mature to produce two seeds within a single spikelet which on germination give rise to two diploid plants.

The authors are grateful to Dr. S. Y. Padmanabhan, Director, C.R.R.I., for his keen interest and for providing facilities for this work.

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#### INHERITANCE OF RESISTANCE TO POWDERY MILDEW (*OIDIUM LINI* SKORIC) IN LINSEED *LINUM USITATISSIMUM* LINN.

THE widely grown crop Linseed is subject to severe infection of powdery mildew caused by *Oidium lini* thus bringing a heavy damage to the crop. A few resistant varieties were identified after screening under severe natural infestation at the J.N. Agri-

TABLE I

Segregation pattern of resistance to powdery mildew infection in linseed

Sl. No.	Cross		No. of plants found to be			Ratio obtained	X <sup>2</sup>	P value	
			Resistant	Susceptible	Total				
1.	E.C. 9832 × Sabour (a)	Observed	195	66	261	3:1	0.115	0.80	0.90
		Expected	195.75	65.25	261				
2.	E.C. 9832 × Hira	Observed	192	65	257	3:1	0.117	0.80	0.90
		Expected	192.75	64.25	257				
3.	E.C. 9832 × No. 55	Observed	165	56	221	3:1	0.136	0.80	0.90
		Expected	165.75	55.25	221				
4.	E.C. 9832 × T. 397	Observed	203	67	270	3:1	0.049	0.95	0.90
		Expected	202.50	67.50	270				
5.	E.C. 9832 × R. 1	Observed	193	66	259	3:1	0.322	0.90	0.80
		Expected	194.25	64.75	259				

cultural University, Jabalpur. E.C. 9832 being one of them was known to possess a high degree of field resistance to powdery mildew<sup>1</sup>.

The studies on the nature of inheritance were undertaken under severe natural infestation in five crosses, viz., E.C. 9832 × Sabour (a), E.C. 9832 × Hira, E.C. 9832 × No. 55, E.C. 9832 × T. 397 and E.C. 9832 × R. 1. Varieties Sabour (a), Hira, No. 55, T. 397 and R. 1 were highly susceptible while the parent E.C. 9832 possessed a high degree of tolerance to powdery mildew. Crosses were made during *rabi* 1971 and the F<sub>1</sub> was studied during *rabi* 1972. The F<sub>1</sub> progenies in all the five crosses and the parent E.C. 9832 were found resistant to powdery mildew. The parents Sabour (a), Hira, No. 55, T. 397 and R. 1 showed the susceptible reaction.

The counts on healthy and infected plants were taken in the F<sub>2</sub> progeny of the crosses grown during *rabi* 1973. In all the five crosses a ratio of three resistant to one susceptible was observed (Table I) indicating the presence of single pair of dominant alleles controlling the resistance in the E.C. 9832 variety. Similar observations on the rust resistance character in linseed made elsewhere have indicated that it was conditioned by a single pair of factors with the resistance being dominant<sup>2</sup>.

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#### FIRST RECORD OF *HELIOTHIS ARMIGERA* (HUBNER) AS A PREDATOR ON THE PUPAE OF CASTOR SEMILOOPER (*ACHOEA JANATA* L.)

DURING the survey of castor fields infested heavily with castor semilooper in Mahbubnagar District (Andhra Pradesh) in the month of November, 1973 the authors came across *Heliothis armigera* Hubner larvae predating upon the pupae of castor semilooper (*Achoea janata* L.). The action of feeding by *Heliothis armigera* H. was exactly similar to its feeding on gram pods (*Cicer arietinum*), viz., the caterpillar was found exposing its posterior half with anterior portion inside eating the pupa of castor semilooper (Fig. 1). During further development in the laboratory the larva consumed one more fresh pupa of castor semilooper and then pupated. The adult emerged after 15 days from the pupa.

Cannibalism in *Heliothis armigera* H. has been reported by Twine<sup>1</sup> (1971) but not predatism. Hence, this is the first record of *Heliothis armigera* Hubner as a predator of the castor semilooper, *A. janata* L.

Thanks are due to Dr. N. G. P. Rao for the encouragement and Dr. Swaraj Ghai, Systematic

Entomologist, Indian Agricultural Research Institute,  
for the identification.

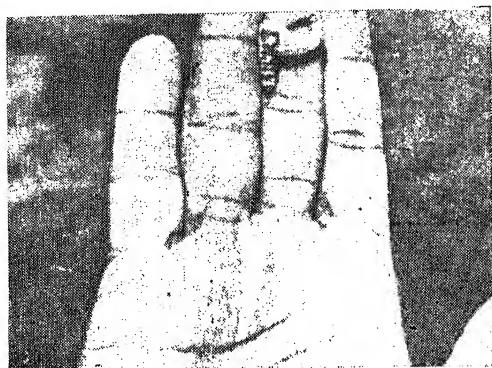


FIG. 1. The larva of *Heliothis armigera* Hubner catching the pupa of castor semilooper (*Achaea janata* L.).

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### EFFECT OF GAMMA RADIATION ON TWO DEVELOPMENTAL STAGES OF ZEBRA FISH EGGS

THE effect of various carcinogens like ethyl carbamate, urethane, 2 acetyl aminoflourene and other chemicals<sup>1,2</sup>, have been studied on fish and other animals. Taguchi<sup>3</sup> worked on the effect of gamma radiation in teleost embryos with special reference to the protective action of 2, 4-dinitrophenol. Gamma radiation has been widely used on algae, woody plants and gymnosperms, mouse placental cells for studying the developmental effects<sup>1,2,4-7</sup>. In the present study, effect of different doses of gamma radiation on two developmental stages of Zebra fish (*Brachydanio rerio*), viz., first cleavage stage and gastrula stage was investigated.

Mated female Zebra fishes were grown in temperature regulated (27° C) breeding nets. One set of eggs was collected within 30 minutes after laying (first cleavage stage) and the other between 12-14 hours (gastrula stage). Three batches of eggs each containing between 70-80 eggs were transferred into scintillation vials containing tap-water. The eggs were maintained in these vials at pH 6-8 at 27° C throughout the experiment. Irradiation was done in Gamma Radiator Model T/0-547 (Picker-

X-ray) containing Cs 137 as gamma source. The timing cycle was started when the drawer was closed into the gamma source with the eggs. Air was supplied with a blower. The gamma source contained hundred Curie of radioactive material, theoretically emitting 3,000 R/hour of gamma-rays.

The radiation was also measured by dosimetry. The dosage of radiation was calculated from the duration of irradiation, using the standard value referred above. Three different doses, 1,500 R, 3,000 R and 4,500 R were used. After irradiation, the eggs were observed at intervals of 15-30 minutes, under the microscope fitted with camera to photograph the developmental sequences by time lapse photography.

Figure 1 shows the per cent mortality of eggs irradiated at the beginning of 1st cleavage stage. Eggs irradiated at 1,500 R developed to a maximum of 20 hours while those exposed to doses of 3,000 R and 4,500 R died at 12 hours. Though no major abnormality was noticed in the developmental stages of irradiated eggs at first cleavage stage, there was considerable time lag in cell division and differentiation. The first cleavage of 1,500 R eggs was completed after 90 minutes as against 45 minutes in the control. The 1,500 R eggs attained development only upto the high blastula stage before disintegrating at 20 hours. As compared to this, the untreated eggs were in the blastoderm stage and the yolk space was half enveloped at 8 hours.

Taguchi<sup>3</sup>, working on gamma irradiation in teleost (*Oryzias latipes*) embryos, found decrease in embryological differentiation as a consequence of lowered physiological activity. He has found that 2, 4-dinitrophenol in concentration of 10<sup>-4</sup> M exerted a protective effect on the developmental disturbances in irradiated embryos at the first stage.

Per cent mortality in the irradiated eggs at the gastrula stage are presented in Fig. 2. Mortality of eggs was low as compared to those irradiated at 1st cleavage stage. Almost 50% of the eggs completed full development (i.e., fish hatched from the eggs). Per cent mortality between 1,500 R and 3,000 R irradiations was not significantly different (44-46%), as compared to untreated. Highest mortality was in 4,500 R eggs (54%). In all the irradiated eggs the development was slower. Fully developed fish hatched out at 94 hours in the untreated. As a contrast 1,500 R eggs were in 32 somite stage only with the absence of retinal pigments and the ventricles of the brain. 4,500 R eggs hatched after 128 hours with conspicuous absence of xanthophores.

On comparison of the two sets of data of irradiated eggs (Figs. 1 and 2), it will be rational

to conclude that eggs at earlier stages of development are more sensitive to gamma radiation than at later stages. Present data support the view that the factors associated with cell division are much more sensitive than those associated with differentiation. Similar results have been reported by Renne<sup>5</sup> and Mau *et al.*<sup>8</sup>. It may be reasonable to conclude that Zebra fish eggs show high radio resistance for gamma radiation at later stages of development.

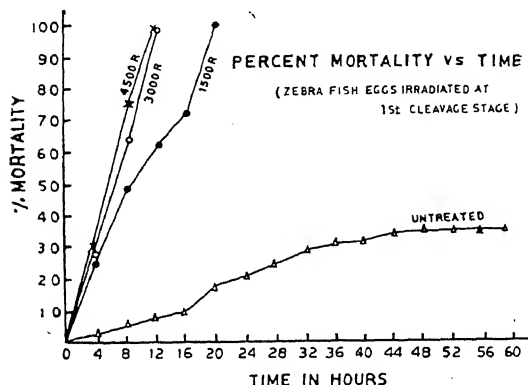


Fig 1

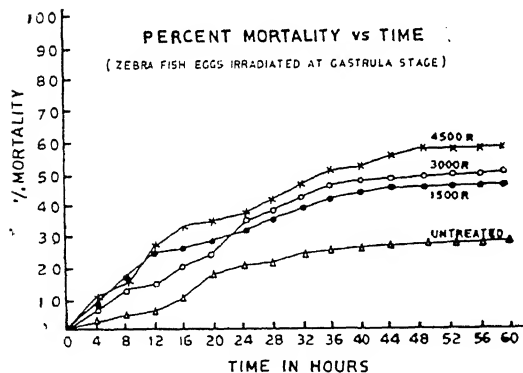


Fig. 2

The author is grateful to Dr. James D. Graham of the Bowling Green State University, Ohio, U.S.A. for helpful suggestions and to Dr. (Mrs.) M. K. Krishnakumari for going through the manuscript.

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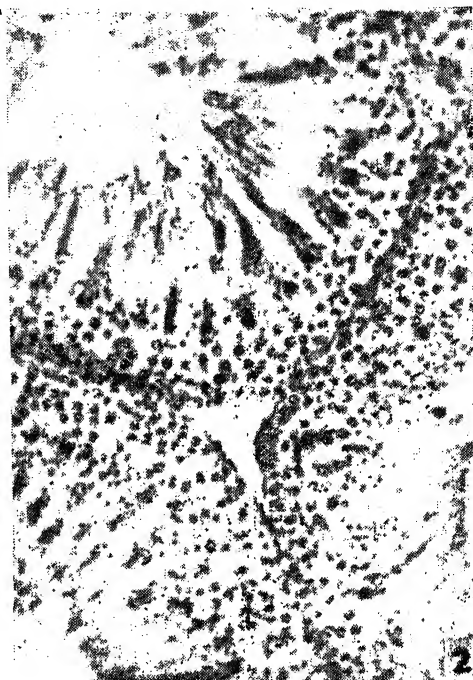
#### SINGLE TESTIS IN THE COMMON HOUSE CROW (*CORVUS SPLENDENS VIEILLOT*) AND BANK MYNA (*ACRIDOTHERES GINGINIANUS LATHAM*)

CRYPTORCHISM is known to occur either temporarily or permanently among mammals<sup>1</sup> but unigonadism has not been reported in mammals or in truly-breeding avian species. However, absence of testes or presence of a single testis has been reported in the offspring of genetic crosses of pigeon. Riddle<sup>2</sup> has described in such 'hybrid' pigeons sixteen cases in which there was no testis at all and in another additional group of sixteen males a single testis was present. In nature, however, among true-breeding avian species such gonadal abnormalities are probably rare and to the best of my knowledge have not been reported.

While studying the annual reproductive cycle of some birds of semi-arid and arid tracts of Rajasthan, one specimen each of the bank myna and the common house crow was encountered which possessed only a single testis. These abnormal specimens were obtained out of the minimum number of at least one thousand birds of each species examined from the wild. In both the abnormal specimens of the bank myna and the common house crow only the left testis with the accessory duct was present while the right testis along with the vas deferens was totally absent (Figs. 1, 3).

In the bank myna the specimen was obtained during the breeding season. The breeding season of the bank myna is from May to August and in the remaining months the birds remain sexually inactive. The unigonadal male specimen was obtained from the wild in the month of June. The single large testis resembled in every respect the testes of normal active males dissected on the same day in gross morphology, size, weight and also histology (Fig. 2).

The breeding season of the common house crow in Rajasthan is from mid of June to mid of August; the birds remain sexually inactive during the rest of the year. The abnormal crow was dissected in the month of January. In this bird spermatozoa were absent in the testicular smear and even the histology of the testis showed all the



FIGS. 1-4. Fig. 1. Reproductive tract of abnormal male bank myna showing only left testis during the breeding season; the right testis being absent (scale in mm). Fig. 2. Photomicrograph of the left testis of the abnormal bank myna specimen showing large number of sperms and other developmental stages,  $\times 200$ . Fig. 3. Reproductive tract of abnormal male common house crow showing only left testis during non-breeding season, the right testis being absent (scale in mm). Fig. 4. Photomicrograph of the left testis of the abnormal common house crow specimen showing large number of interstitial cells,  $\times 200$ .



inactive stages (Fig. 4) as similarly noticed in the normal male birds autopsied on the same day. In gross size and weight the unilateral testis compared well with those of the testes of normal crows.

While considering the above two abnormalities in the male reproductive organs of the bank myna and the common house crow, I may be right in assuming that such abnormalities occur not only among hybrids but in nature also. This type of testicular abnormality appears to be purely developmental in which physiological rather than cytological or genetic changes are concerned.

I am grateful to Prof. L. S. Ramaswami for guidance and encouragement during the course of this work.

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#### A NEW BRACHIONID ROTIFER *PLATYIAS QUADRICORNIS ANDHRAENSIS* SUBSP. NOV. FROM INDIA

IN India, *Platytias quadricornis* Ehrenberg, 1832, has been recorded from Calcutta by Anderson<sup>1</sup>, from Srinagar and Ladakh by Edmondson and Hutchinson<sup>2</sup>, from Kerala by Nayar and Nair<sup>3</sup> and from Andhra Pradesh by Dhanapathi<sup>4</sup>. During a limnological investigation five specimens with unique characters, apart from specific ones, were obtained in May 1971, from Hussain Sagar reservoir in Hyderabad (A.P.). At the time of collection, temperature of the water was 26° C and pH 7.6:

**Description.**—Lorica pear-shaped, firm and compressed antero-dorsal margin provided with two stout spines bending ventrally and tapering into acute points; posterior spines longer than the anterior, with swollen bases and tapering into claw-like acute points. Lorica tuberculated with regular facets. Foot jointed and nonretractile; proximal region of the first joint girdled by characteristic cuticular plates; toes long, spindle-shaped and ending in points (Fig. 1).

The specimens measured 280  $\mu$ –308  $\mu$ , in total length, with a maximum width of 238  $\mu$ –252  $\mu$ . Measurements of principal parts of largest specimen are: length of anterior spines 70  $\mu$ , of posterior spines 98  $\mu$ , of toes 56  $\mu$ ; width at anterior end of lorica 140  $\mu$  and distance between posterior spines 98  $\mu$ .

**Remarks.**—It agrees with descriptions of *P. quadricornis* in having tuberculated lorica with

regular facets, jointed and nonretractile foot and antero-ventral margin without spines, but differs in having a large pear-shaped body, anterior spines ending in acute points, longer posterior spines with swollen bases and claw-like points, and in having base of first foot-joint girdled by characteristic cuticular plates.

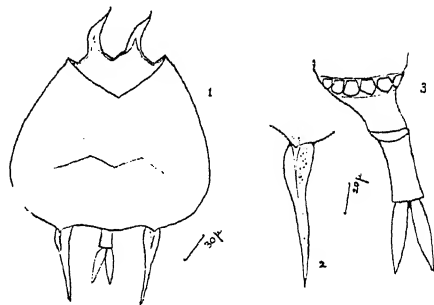


FIG. 1. 1, *Platytias quadricornis andhraensis* subsp. nov.; 2, Posterior spine; 3, Foot and toes.

Ahlstrom<sup>5</sup> states that the lengths of toes and of anterior and posterior spines of *P. quadricornis* are variable. He recorded a small variant along with a large form having a width of 225  $\mu$  and with posterior spines measuring 80  $\mu$ , without any evidence of intergradation, based on collections from Florida. Interestingly, the present large specimens occurred without any evidence of intergradation, exhibiting new characters apart from the difference in maximum length. The claw-like acute points of the posterior spines, and cuticular plates at the base of the first foot-joint are distinctive characters. Hence the large variant is considered a new subspecies: *Platytias quadricornis andhraensis*.

Thanks are due to Mr. S. Rama Rao, Head of Department of Zoology, D.N.R. College, Bhimavaram, for providing necessary facilities to carry out the work.

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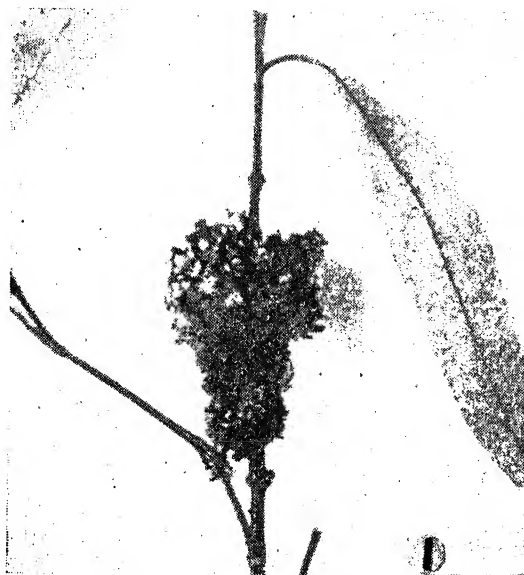
**GALL FORMATION BY *PUCCINIA THWAITESII* BERK. APUD BERK. AND BR. ON *GENDARUSSA VULGARIS* NEES.**

AN interesting case of gall formation in *Gendarussa vulgaris* Nees, caused by *Puccinia thwaitesii* Berk. apud Berk. and Br. was observed during October, 1973 from Trichur in Kerala State. A survey of the literature reveals that so far a case of this fungus inducing gall formation in plants is unknown, though it has been reported in connection with the leaf rust in the same host<sup>1,2</sup>. An instance of gall formation in *Urtica parviflora* caused by *P. urticae* has been studied by Rao<sup>3</sup>. The only report of *Puccinia* causing gall formation of the whole shoot is that of Mani<sup>4</sup> on *Crisum arvense* Scop. by *P. suaveolens* Pers.

formation in such galled shoots were also noticed. These roots are thicker than the normal ones and possess swollen bases (Fig. 2).

The attack of the fungus on the axillary bud brings about increased cellular activity—division and enlargement of cells of the corpus layer—as a result of which the zonation pattern of the apex gets disturbed. The development of leaf is found to be arrested at the primordial stage and new meristematic centres arise not from the axil of the infected leaf, but slightly deviated from the normal axial position.

The authors are grateful to Dr. B. K. Nayar, Prof. and Head of the Department of Botany for encouragement and for providing the necessary facilities. One of us (P. N. U.) thanks the Univer-



FIGS. 1-2. Fig. 1. A galled shoot showing the the nodes ( $\times \frac{1}{4}$ ). Fig. 2. A galled shoot showing ( $\times \frac{1}{4}$ ).

clustering of galls at the axillary position around roots with swollen bases arising from the node.

In nature, the galls originate from the infected dormant axillary bud. This brings about the swelling of the nodes and shortening of the internodes, thereby giving a stunted appearance to the galled shoot. Normal and deformed leaves may arise very close to each other due to the extremely short internodes of the dwarfed shoot. Later, profuse proliferation of the axillary buds of the galled shoot takes place and these galls cluster around the nodes which simulates an inflorescence (Fig. 1). The leaves arising from such clusters are thicker, narrow and smaller than the normal leaves. Pustules of this fungus are visible as black spots throughout the galled shoots and leaves. Generally, the terminal bud is found to be free from infection. At maximum severity, the galled shoots get killed. Aerial root

sity of Calicut for the award of a Research Fellowship.

Department of Botany,  
University of Calicut,  
673 635, Kerala,  
March 22, 1974.

P. N. UNNI,  
V. J. PHILIP.

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3. Rao, C. G. P., *Indian Phytopath.*, 1971, 24, 756.
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## SHORT SCIENTIFIC NOTES

*Diplodia* Die Back of Nutmeg

Twigs and branches of nutmeg (*Myristica fragrans* Hoult) trees growing in the Gokul Estate at Vithura, Trivandrum District, were found drying during January, 1972. The leaves of affected shoots became brown, rolled and dried. The drying and death of leaves and shoots extended back, involving larger branches. The fruits on affected branches also became brown, shrunken and dried.

Laboratory examination of dried twigs placed in moist chamber revealed the presence of numerous pycnidia of *Diplodia* sp., which was subsequently identified as *Diplodia natalensis* Evans. The pathogen was isolated and brought to pure culture on oat agar medium on which it sporulated well. The unicellular, hyaline pycnidiospores measured  $16.30$  to  $22.82 \mu \times 11.43$  to  $14.67 \mu$ , while the two-celled, dark brown spores measured  $14.30$  to  $20.16 \mu \times 9.78$  to  $13.00 \mu$  in size. Pathogenicity of the fungus was tested and proved by artificial inoculation. The pathogen could infect fruits of nutmeg, orange and snake gourd, when inoculated in the laboratory. Abundant pycnidia and spores were produced on nutmeg and snake gourd fruits.

Ramakrishnan and Sarojini Damodaran<sup>1</sup> reported a fruit rot of nutmeg caused by *D. natalensis* from Burliar, Tamil Nadu. There is no record of this fungus causing die back of nutmeg trees in India.

Based on the studies on *Diplodia* dry rot of guava fruits<sup>2</sup>, 0.3% ziride was sprayed on affected trees after pruning the shoots showing die back, well below the infected region and was found to be effective in controlling the disease.

The authors wish to thank Dr. J. Sam Raj, Dean, Faculty of Agriculture, Kerala Agricultural University, for encouragement.

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Pampadumpara,  
Idikki District,  
Kerala State,

and  
College of Agriculture, P. K. SATHIARAJAN,  
Vellayani, Kerala State,  
February 28, 1974.

1. Ramakrishnan, T. S. and Sarojini Damodaran. A. P., *Indian Phytopath.*, 1954, 7, 7.
2. Rajagopalan, B. and Wilson, K. I., *Agric. Res. J. Kerala*, 1972, 10, 194.

Some Additions to Indian Fungi: Some New *Acremonia*

In an earlier communication<sup>1</sup>, as many as eight species of imperfect fungi, isolated from the rhizosphere of some medicinal plants of this area, were reported as new records from this country. The list included a single species of *Acremonium*, viz., *A. killiense* Grutz. Further studies have yielded three other *Acremonium* spp. A scrutiny of the list of Indian fungi in recorded literature indicated that all the three species isolated by the author were new records from the country. Method of isolation<sup>2,3</sup> and culture media employed were the same as indicated earlier<sup>1</sup>. The fungi along with the name of the plants from which isolated are tabulated below:

Name of fungi	Plant from which isolated
1. <i>Acremonium strictum</i> W. Gams (IMI No. 168807)	<i>Argemone mexicana</i> L.
2. <i>A. sclerotigenum</i> (F. and R. Moreau ex Valenta) W. Gams (IMI No. 168854)	<i>Rauwolfia serpentina</i> (L.) Benth. ex Kurz.
3. <i>A. potronii</i> Vuill (IMI No. 168847)	<i>Evolvulus nummularius</i> L.
*4. <i>A. killiense</i> Grutz (IMI No. 163122)	<i>Cassia occidentalis</i> L.

\* Reported in the earlier communication also.

All these fungi have been deposited in the Commonwealth Mycological Institute, Kew, England.

The author gratefully acknowledges the guidance and the laboratory facilities extended by Professor K. S. Bilgrami. Sincere thanks are also due to Mr. A. Johnston and Dr. Hawksworth, C.M.I., Kew, England, for confirming some of the identifications.

P.G. Department of Botany, R. N. VERMA,  
Bhagalpur University,  
Bhagalpur-812007 (Bihar),  
February 24, 1974.

1. Bilgrami, K. S. and Verma, R. N., *Curr. Sci.*, 1972, 41, 504.
2. Waksman, S. A., *Principles of Soil Microbiology*, Bailliere Tindall and Co., London, 1927.
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**Incidence of Scarlet Mite, *Brevipalpus phoenicis* (Geij.) on Sesamum in West Bengal**

Recently, while surveying the mites associated with oilseeds of West Bengal during August 1972, *Brevipalpus phoenicis* (Geij.) a new mite pest of sesamum (*Sesamum indicum*) in this State was noticed. This mite is commonly known as scarlet mite because of its colour and has been reported earlier in India on tea<sup>1</sup> and rose<sup>2</sup>.

All stages of this mite were seen on the under-surface of leaves and on the upper surface as well (in the case of heavy infestation). Because of continuous sucking the plant sap, light brownish spots appeared at the points of feeding which later turned light to deep brown. All the attacked leaves became twisted and folded longitudinally. Raised gall-like swellings appeared in some of the infested leaves but whether these were caused because of

feeding of this mite is not known with certainty. Besides leaves, they also attacked the young twigs and pods which turned reddish brown and looked sickly.

One species of thrips and a species of *Amblyseius* were found feeding on all the stages of this mite.

The author is grateful to Dr. A. P. Kapur, Director, Zoological Survey of India, Calcutta, for the facilities.

Zoological Survey of India.

S. K. GUPTA.

34, Chittaranjan Avenue,

Calcutta-12, February 11, 1974.

1. Das, G. M., *Two and a Bud, Cinnamara*, 1961, 8, 3.
2. Ghai, Swaraj and Menon, M. G. R., *Entomologist Newsletter*, 1970, 1, 37.

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## REVIEWS AND NOTICES OF BOOKS

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**Hadron Physics at Very High Energies.** By David Horn and Fredrik Zachariasen. (W. A. Benjamin, Inc., Advanced Book Program, Reading, Massachusetts), 1973. Pp. xvii + 378. Price \$ 17.50.

The above book under review appears in the lecture note and reprint series. The authors have discussed the currently intriguing problems that crop up in the interactions of hadrons at very high energies. It was expected that asymptotic regime would be attained at energies up to a few GeV. But many complicated structures appear in the cross-sections and angular distributions, etc. The expectation that the cross-section will become asymptotically flat near a few hundred GeV has not been realised yet. The authors review the existing experimental results and discuss the various theoretical models and analyses. These include the Regge pole analysis, field theoretical and multiperipheral models and various other models such as the droplet model, statistical models, diffraction models and hybrid models. These discussions are helpful for understanding the fundamental theoretic basis of high energy physics. However, they have not included the hadron model and the dual models. The reason given is that these give only qualitative results and the latter does not represent Pomeron effects.

There are five appendices which give mathematical details of some useful theorems.

To sum up the book has appeared in time and will be helpful to those doing research in very high energy physics. Since it is addressed to research

workers in this field, the level is rather advanced. The reviewer feels that the book will be valuable for active research workers in this field.

K. P. SINHA.

---

**Basic Principles of Plasma Physics: A Statistical Approach.** By S. Ichimaru. (Addison-Wesley/W. A. Benjamin, Inc., Advanced Book Program, Reading, Massachusetts 01867), 1973. Pp. xviii + 324. Price \$ 19.50.

This is one of the latest books in the subject. Therefore one expects it to contain the latest developments in the statistical methods of plasma theory. In this regard the author has done only a partial job, otherwise, the book is well written. Though, some chapters contain only the usual stuff that can be found in other books like *Plasma Kinetic Theory* by Montgomery and Tidman, but chapters 9 to 11 on Fluctuation, relaxation, and plasma turbulence have been written with a lot of care and understanding, even though here the reader may find some overlap with 'Methods of Nonlinear Plasma Theory' by Davidson and 'Fluctuation in Plasmas' by Sitenko. On page 278, one finds three-wave interaction represented by a diagram of many body quantum theory, but hardly any treatment of the technique is found in the entire book except in Appendix E that, incidentally, contains only one reference that of Pines. Reference to other works in the area as applied to plasma theory of thermonuclear interest is missing.

Over all the book is a positive contribution and not merely another book in the ever-increasing area of book publishing in Plasma Science.

K. P. SINHA AND S. KRISHAN.

The book will be a useful addition to an undergraduate Mathematics library.

R. VITTAL RAO.

### ANNOUNCEMENTS

#### Institution of Chemists (India)—Associateship Examination 1975

The twenty-fifth Associateship Examination of the Institution of Chemists (India) will be held in November, 1975. The last date for Registration is 30th November, 1974.

The Examination is recognised by the Government of India as equivalent to M.Sc. in Chemistry for purposes of recruitment of Chemists.

Further enquiries regarding this and for Membership may be made to the Honorary Secretary, Institution of Chemists (India), Chemical Department, Medical College, Calcutta-12.

#### IV International Palynological Conference

The Fourth International Palynological Conference will be held at Lucknow (India) from 29th December 1976 to 5th January 1977. The First circular was issued in April 1974. Those who have not received the circular but wish to receive it, may kindly write to The Secretary-General, IV International Palynological Conference, 53 University Road, Lucknow-226007.

#### Award of Research Degrees

The M.S. University of Baroda has awarded the Ph.D. degree in Physics to Shri K. K. Gopinathan Menon for his thesis entitled "Ultrasonic Studies of Metals and Alloys (Bismuth and some of its Alloys)"; Ph.D. degree in Zoology to Shri R. Govinda Panickar for his thesis entitled "Histophysiological Studies on Developing and Adult Avian Gizzards"; Ph.D. degree in Microbiology to Kumari Dharmdasi Chinubhai Patel for her thesis entitled "Metabolic Fate of  $\beta$ -hydroxy-butyrate in Rhizobial Species".

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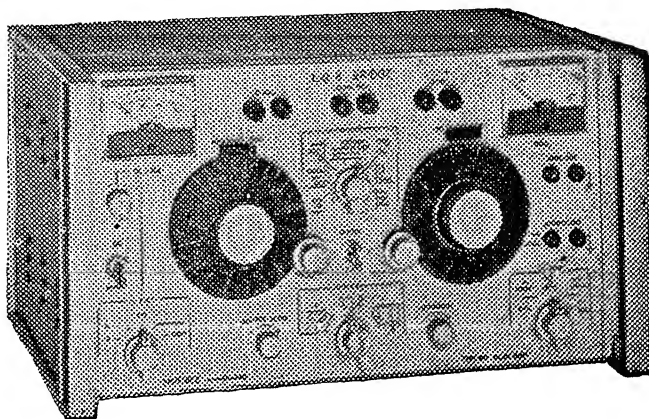
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## TRENDS IN BOTANICAL THOUGHT AND RESEARCH : IN RETROSPECT AND IN PROSPECT

F. C. STEWARD, F.R.S.\*

*Sir C. V. Raman Visiting Professor, University of Madras*

THE pursuit of knowledge through science, as indeed other human activities, has its moods and its fashions, its high periods and its depressions. Plant science also has been shaped by a series of such trends and it is a useful exercise to see them in retrospect and anticipate them in prospect. An excuse for the writer to do so is the fast approaching 50th Anniversary of his own entry, after graduation in chemistry, into botanical research. This exercise is also prompted by the recurrent question, repeatedly posed during a brief sojourn in India, how that so diverse country with its ancient traditions and great problems should adapt its scientific efforts (particularly in biology and botany) to the current scene.

The origins of botany and plant physiology are as ancient as the arts of agriculture and the use of plants in primitive medicine. One may, no doubt, still search for the origins of this, or that, botanical thought or principle in the ancient history and writings of India as in the culture of any other great centre of human development. Because the most modern developments of science have been so identified with the West, where the dramatic events of the XIXth century, with its wars and industrial revolution, paved the way for the accelerated scientific pace of the XXth century, most students have seen the history of botanical science in western terms. Following the histories of Sachs (1875) and of Reynolds Green (1909), the time course has been traced from Aristotle and Theophrastus to arrive, tortuously, *via* Van Helmont, John Woodward, Malpighi, Stephen Hales, Joseph Priestley, de Saussure at the turn of the XIXth century. Thereafter, the great procession of the XIXth century includes such names as Robert Brown, Nageli, Mendel, Pasteur, Hofmeister, De Bary, Sachs, Strasburger, Pfeffer, De Vries, Haberlandt. But in any search for ultimate origins, problems still arise. The oft-quoted first plant physiological experiment of Van Helmont (1577-1644), even the first recorded experiment in nutrition and natural science, tested an idea that was in conflict with Aristotelian doctrine but was as clearly expressed 150 years before Van Helmont by Nicholas of Cusa (1401-1464) though it was not attributed to him by Van Helmont. It is an interesting question whether ancient Sanskrit manu-

scripts contain similar, or even earlier, references to such basic nutritional concepts.

But the XIXth century saw the dichotomous development of the interpretation of plants based on descriptive observations of their characteristics (through taxonomy, morphology, histology and cytology) and analytical interpretations of their functions (through physiology and biochemistry) based on a rational system of chemistry. It is a strange paradox that the monumental works of the XIXth century morphologists, that laid the foundations of botanical science, are now so often ignored, or even disparaged, in the search for causation in purely physical and chemical terms. It is a paradox because the physiological functions about which so much seems to be known in terms of the chemical steps by which they occur (*e.g.*, protein metabolism and synthesis; carbohydrate metabolism, respiration and photosynthesis; the uptake and accumulation of ions from dilute solution) still require that essential setting in the cell systems in which they occur in order that they may proceed at the pace, or with the efficiency, and under the requisite degree of control that the conditions *in vivo* demand. On the current scene no plant physiologist or biochemist can really afford to ignore structure and organization; he must in short be concerned with morphology.

The first hope and lesson is that students in India should still be taught the importance of perceptive visual and descriptive observations and that great insights may often flow from simple experiments. Those who, too soon, become the slave of their purchased equipment, or who regard its lack as a total barrier to all progress, may well miss the challenge that has set so many in the past on an original voyage of discovery. Pioneers like Stephen Hales, Priestley, Mendel, Darwin and others improvised and relied upon their powers of observation and interpretation—they were not fettered by slavish adherence to any currently acceptable school of thought or the acceptable kind of experimental approach.

But no current 'fashion' should be allowed to become arrogant and overbearing—to claim to be recognised as the sole respectable avenue to truth and understanding. So many fashions or fads of the past have flourished in their day until their limitations became apparent. The rise of cell physiology and the early thoughts that a knowledge of the composition and chemical substances of cells

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implied that this would lead to understanding, illustrates this point. In the 1920's people still wrote and thought about protoplasm as a material substance (not as an organisation), about its physical and chemical properties. But an early faith in the chemistry and chemical composition of cells *per se*, and in the application of the physics and chemistry of the day, which necessarily related to equilibria, soon gave way to the doctrines of General Physiology and the then 'new' thoughts that the meaning of life would emerge through knowledge of those properties that cells and their protoplasts had in common. General Physiology soon merged into General Cytology with its attention to membranes and cellular inclusions and later to the staining properties of self-reproducing organelles. Meanwhile, the advancing knowledge from protoplasm as the 'physical basis of life' to the nucleus and the chromosomes as 'the physical basis of heredity' laid the foundations for those borderline developments between cytogenetics and biochemistry that have later proliferated under the label of 'molecular biology'. But the doctrines of Comparative Biochemistry and of Biochemical Cytology each represented sequential 'fashions' along the way. And though the term 'Molecular Biology' really dates back to Astbury in the early 1930's, for whom it was really the molecular configuration (*i.e.*, architecture) of biologically important large molecules, it has come to mean the working out of the importance and role of the nucleic acids in the transfer and maintenance of genetic information and the means by which that information is translated into action through its ability to regulate biosynthesis and metabolism. There are even those today who go so far as to believe that no modern study can be worthwhile unless it has its component of 'molecular biology'.

But dramatic as the so-called Molecular Biology developments have been and even as the Nobel awards have proliferated, one can see clearly the limitations even of this latest, and most dramatic 'fashion'. Although widely hailed as the 'new biology', that is credited with being the wave of the future in the XXIst century, it is still very far from being a substitute for the 'old biology'.

Although molecular biology tells us much about how genetic information is uniquely established in the zygote of a sexually reproduced organism and how it is retained through successive equational divisions throughout development, it has yet to reveal the secrets of development, *i.e.*, how the persistent information in the DNA of the nuclei is so controlled and programmed that it 'tells' the cells what to do when and where they should. And

this is so despite the elaborate, largely semantic, exercises by which we are told that this occurs.

In other words, morphology and embryology and anatomy (pursued experimentally with the modern tools of phase and electron microscopy into the fine structure of cells) are not, though essentially descriptive, outmoded; they are, in fact, as essential as ever if the 'new' or 'molecular biology' is ever to yield up the answers to the problems of development. But these problems of development and morphogenesis cannot be studied only on the simplest systems—one can hardly understand an oak tree or a palm with their organised growing regions and their elaborate morphogenetic responses, by work done only on *Escherichia coli* or bacteriophage. Therefore, one hopes that the Universities and especially the new centres of India will not put the bulk of their resources into slavish imitations of what has been done in the West since the end of World War II. Like so many earlier trends and doctrines that of 'molecular biology' may already have yielded its major returns.

This then is a plea for the maintained excellence of morphology, embryology and the study of morphogenesis—areas which in the past have both well developed in India. With a rich flora upon which to draw, containing many economically important plants about the growth and development of which so little is known, Indian scientists can describe the setting in which the principles of molecular biology can be intelligibly applied. But they may also show—unwelcome as this thought may be to some—that the morphological setting, through 'messages' yet to be deciphered may well control how the genetically prescribed biochemistry shall work. It may be currently fashionable to assume that cells must at all times 'ask their nuclei' for permission to carry out this or that event. But surely the visible evidence of morphogenesis suggests that once the unique genetic information is conferred and is conveyed by successive equational divisions that cells, especially the 'totipotent' cells of higher plants, already 'know' how to do everything that is required of them. In fact, it is their morphological setting, which mysteriously 'tells' them how to use their innate information; not it seems gene by gene, activating or deactivating them, but bringing in *en bloc* large combinations of characteristics which link form and biosynthesis and metabolism. We seem neither to know how these comprehensive signals are given or how they are collectively perceived and rendered operative. In this sense, therefore, morphology and the environment or milieu of cells, as in the organised growing points of higher plants, 'calls the shots' and the biochemistry duly responds.

Thus, it is regrettable how over-anxious some young scientists, in India or elsewhere, seem to be to imitate the latest molecular biology tricks they have seen or heard about, often when abroad, and how they crave the latest in equipment by which to follow these latest fashions. Too often the current trend seems to be that we already 'know the rules' and nature is, as it were, being coerced to obey them. How much more rewarding it may be to recognise that even the new biology, conceived in molecular terms and in the language of the nucleic acids, may not yet have all the answers to the great problems of development, differentiation and morphogenesis? Here surely the role of investigators should be reversed. Instead of, as it were, prematurely dictating to nature in molecular terms, they should be listening in; 'tuning-in' by observation on those continuing 'dialogues' between nucleus and cytoplasm, between adjacent cells in their immediate milieu, between tissues and organs of the plant body as they determine and regulate 'divisions of labour', and between the organized growing regions of plants as they respond to environmentally induced messages, however they are perceived and transmitted into effect. So much that is to be done to sketch in this rich background of form and function, of stimulus and response, of environmental controls of metabolism, nutrition and morphogenesis threatens to succumb to the mistaken search for proofs of preconceived simplistic unitary explanations for the responses of essentially complex and non-equilibrium systems which are affected by so many variables and their multiple interactions. In fact, experimental plant physiology now needs those who are willing to work out devices for studying the interactions of so many simultaneously operating variables rather than attempting to study them singly in isolation.

One therefore hopes for a resurgence, in modern terms, of the older disciplines of morphology and anatomy with the use of all the tools (from hand lens to electron microscope) to render visible the events along the way from zygote to organism. One looks also for a resurgence of interest in the behaviour of plant growing points as they respond morphogenetically to the varied causal factors that control development. And the study of metabolism and biosynthesis, aided by the technical advances of all forms of chromatography and radioautography should not merely be thought of as an appendage to the role of genes in action, or seen in the image of a depressing static metabolic chart on a laboratory wall, but as one of the ways in which one can see the multiple interactions at work between

environments and nutrients and organized cells and growing regions during development.

However, the pursuit of these problems, using many plants (some economically important which have not yet become stereotyped as laboratory objects) can be and should be adapted to the scale of resources available and to the level of investigation needed at the outset.

Would Darwin's Power of Movement in Plants or his Insectivorous Plants or Pollination in Orchids, or Mendel's work on peas, ever have emerged to change the trend of thought of the times unless the enquiring mind of a keen observer had addressed itself to the evident problem at hand? Of course, the time comes when, to pursue the analogy, the studies of Darwin on coleoptiles need the sophisticated studies of the biochemistry of growth factors and of their modes of action and the work of Mendel on peas, proliferating through that of others on *Drosophila* and *Zea mays*, etc., leads to and requires the sophisticated studies of nucleic acid structure and protein biosynthesis. But the point is that in each period and in each age there must be those who are not merely content to "follow the fashion" but who have the initiative and insight and curiosity to open up new areas as they seek, often by simple means, new light on how nature works.

Therefore, in the last third of the XXth century, building upon the great advances in cell physiology, biochemistry and genetics, there is both a great opportunity and a great challenge to determine how development works; in effect to know how nature, having elaborated rigorous mechanisms to preserve genetic identity, produces within this system such great diversity during development. In meeting this challenge botanists everywhere, and specifically in India, have a great and thrilling opportunity if they will adapt their enquiries to their resources, their experimental approaches to their problems rather than searching for problems that fit well-stocked laboratory or its pre-purchased sophisticated equipment.

In my humble experience the best ideas which have turned the course of events emerged often from experiments done simply—however sophisticated the later means to pursue them to finality became. This personal message to botanists and biologists in India is but a poor return for the stimulating experience of being Sir C. V. Raman Visiting Professor in the University of Madras but, having re-read the account of Raman's life and work, I would like to think that, in some respects, he might have approved.



A SEMIPHENOMENOLOGICAL THEORY OF SUPERFLUID LIQUID  $^3\text{He}$ 

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## ABSTRACT

It is shown that the recently observed two phase transitions, namely, A (Second order) and B (First order) in liquid  $^3\text{He}$  below 3 mdeg K can be understood semi-quantitatively in terms of an effective spin Hamiltonian, incorporating an intra-pair and an inter-pair spin dependent interactions. We assume BCS type pairing with 1-odd ( $S = 1$ ) in phase-A and 1-even ( $S = 0$ ) in phase-B. It is shown that the experimental observations are in fair agreement with the theory.

RECENT experimental observations<sup>1-3</sup> show that liquid  $^3\text{He}$  undergoes a second order phase transition at 2.65 mdeg K (Phase-A) and a first order transition at 2.0 mdeg K (Phase-B). Possibility of the new phases being of superfluid nature, with pair condensation in higher angular momentum states ( $1 > 0$ ) has been suggested<sup>4,5</sup>. However, no explicit model Hamiltonian describing the two phase transitions and the physical properties of the two phases, has so far been suggested.

In this paper, we propose a simple model Hamiltonian which seems to describe these phases fairly well, in terms of an intra-pair and an inter-pair spin-dependent interaction. We take cue from the fact that the transitions appear to involve the dynamics of the nuclear spins in an essential fashion, in the reciprocal space.

We write the effective spin Hamiltonian phenomenologically, as

$$H = \Delta \sum_{\vec{k}} \vec{s}_{\vec{k}} \cdot \vec{s}_{-\vec{k}} - \frac{D}{N} \sum_{\vec{k} \neq \vec{k}'} S_{\vec{k}}^z S_{\vec{k}'}^z - H_0 \sum_{\vec{k}} S_{\vec{k}}^z \quad (1)$$

with the total spin  $S_{\vec{k}} = \vec{s}_{\vec{k}} + \vec{s}_{-\vec{k}}$  of spins of the nuclei paired in the time reversed states  $\vec{k}, -\vec{k}$ . The summation is restricted to a phase space shell (of width  $\delta$ ) around the Fermi surface.  $\Delta$  is the intra-pair excitation energy required to go from a spin singlet ( $S_{\vec{k}} = 0$ ) to a spin triplet state ( $S_{\vec{k}} = 1$ ), along with the concomitant orbital excitation to preserve overall antisymmetry. Note that the Hamiltonian contains the intra-pair spin transition and also the cooperative feature of the spin pairs [first and second terms of eq. (1) respectively].

The partition function for the Hamiltonian in eq. (1) can be evaluated exactly. We have,

$$Z = T_r \{ \exp(-\beta H) \} = 2 \sqrt{N} g_1 \int_{-\infty}^{\infty} dx e^{Nf(x)} \quad (2)$$

with,

$$f(x) = -\pi x^2 + \ln \left\{ \frac{1}{2} \left( \frac{1}{g} e^{\beta \Delta} + 1 \right) + \cosh(\beta H_0 + \sqrt{4\pi} \beta D x) \right\},$$

where  $g = g_1/g_0$  is the ratio of the orbital degeneracies in the triplet ( $S=1$ , 1 odd) and the singlet ( $S=0$ , 1 even) states. In deriving eq. (2) we have used the Stratonovich identity,

$$e^{a^2} = \int_{-\infty}^{\infty} dX e^{(-\pi x^2 + 2\sqrt{\pi} ax)} \quad (3)$$

to linearize the term  $D \left( \sum_{\vec{k}} S_{\vec{k}}^z \right)^2$  occurring in the

exponent. The evaluation of the integral in eq. (2) is done by saddle point method. The analysis of the free energy in the limit of zero magnetic field and for  $\eta \equiv \Delta/D > 1$  and  $g > 1.45$  shows two phase transitions; a second order phase transition at  $T = T_{ca}$  and another phase transition at a lower temperature  $T = T_{cb}$  which may be first or second order depending on the value of  $\eta$ . The two critical temperatures  $T_{ca}$  and  $T_{cb}$  are given by the roots of the equation,

$$\frac{1}{2} \left( \frac{1}{g} e^{\beta \Delta} + 3 \right) - 2\beta D. \quad (4)$$

For temperatures  $T_{cb} < T < T_{ca}$ , the system shows spontaneous magnetization  $M_s(T)$  per pair, (in units of twice the nuclear Bohr magneton) given by the transcendental equation,

$$M_s(T) = \frac{\sinh(2\beta D M_s)}{\frac{1}{2} \left( \frac{1}{g} e^{\beta \Delta} + 1 \right) + \cosh(2\beta D M_s)}. \quad (5)$$

Following qualitative features can be easily noted. Phase-A is an ordered phase with pairs in the triplet state ( $S=1$ , 1 odd), while in phase-B the pairs are in singlet state ( $S=0$ , 1 even). For the choice of the parameters  $g=3$ ,  $\eta = 1.45$  and  $T_{ca}/T_{cb} \simeq 1.3$ , it is found that the spontaneous magnetization in phase-A increases monotonically with decreasing temperature and falls abruptly to zero at  $T_{cb}$ , the fall being independent of the field and of the same order of magnitude as has been observed<sup>3</sup>. Clearly A transition is second order and B is first order. Also the fall in the

magnetization decreases as we decrease the ratio  $T_{CA}/T_{CB}$  and near the triple point in the P-T phase diagram the B transition becomes of second order, again being consistent with the experimental findings<sup>6</sup>. The NMR frequency shift  $\nu_{liq}^2(T, H) - \nu_{sol}^2(T, H)$  was found<sup>1</sup> to be proportional to  $(1 - T/T_{CA})$  and also independent of H in the phase-A. For zero field case this would imply  $\nu_{liq}^2(T, H=0) \propto (1 - T/T_{CA})$  in the phase-A. The shift vanishes abruptly at B transition. It is found that the solution of eq. (5), for the above-mentioned values of the parameters, gives a linear behaviour of  $M_z^2(T)$  in the phase-A ( $H=0$ ) and an abrupt vanishing at B, confirming the experimental facts. The other experimental observations like the specific heat jumps at A transition and the constancy of the transverse magnetic susceptibility  $[\chi_{\perp}(T)]$  in phase-A and then an abrupt fall in  $\chi_{\perp}(T)$  at B<sup>1</sup> also follow from our theory. The specific heat jump is found to be proportional to D. Adding a transverse field term to the Hamiltonian and ignoring commutators involving the total spins, we get  $\chi_{\perp}(T) \simeq 2/D$ , a constant in the phase-A,

and at the B transition a discontinuous drop given by,

$$\begin{aligned} \frac{\chi_{\perp}(T \rightarrow T_{CB}^-)}{\chi_{\perp}(T \rightarrow T_{CB}^+)} &\simeq \frac{D}{k_B T_{CB} \left[ \frac{1}{g} \exp(\Delta/k_B T_{CB}) + 3 \right]} \\ &\simeq \cdot 25. \end{aligned} \quad (6)$$

In conclusion, we have been able to explain the anomalous nature of the two phases in liquid <sup>3</sup>He below 3 mdeg K on the basis of an effective spin Hamiltonian with semiphenomenological parameters. Detailed calculations will be published elsewhere.

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## CONCENTRATION OF JAPANESE ENCEPHALITIS (JBE) VIRAL ANTIGENS PREPARED FROM VERO CELL CULTURE BY SUCROSE DIALYSIS

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### ABSTRACT

Concentration by sucrose dialysis of HA and CF antigens of JBE virus prepared from Vero cell culture is described. This method is rapid, reliable, economical and does not require any special equipment or material. Antigens thus concentrated can be employed in serological tests.

### INTRODUCTION

THE production of high titred arboviral antigens from the infected suckling mouse brains is a procedure routinely employed in most of the laboratories working on arboviruses. Clarke and Casal's<sup>1</sup> sucrose acetone (SA) extraction method is most suitable for the preparation of antigens for haemagglutination-inhibition (HI) as well as complement fixation (CF) tests. However, the procedure is rather laborious, potentially hazardous and requires voluminous acetone. The tissue culture system for the production of arboviral antigens is more convenient and less hazardous, but usually the titres of antigens are comparatively poor<sup>2-6</sup>. Several procedures are employed for concentrating viral antigens<sup>4-6,20</sup>. Concentration by sucrose dialysis of HI and CF antigens prepared from Vero cell culture is described in this communication. The Japanese encephalitis virus, a Togavirus, (Andrewes)<sup>21</sup> is employed in the study.

### MATERIAL AND METHODS

Sucrose is commonly used for preparing density gradient. It is hygroscopic and highly soluble in water and has little effect on viruses. Infected tissue culture fluids (ITCF) harvested on different days were dialysed against solid sucrose or saturated sucrose solution and the concentrated antigens were tested for haemagglutination (HA), HI and CF tests to find out the potentialities of antigens for use in the routine diagnostic serological tests.

Growth and maintenance of Vero culture, methods for inoculation of virus JBELP3 (P 20778) and harvesting ITCF are described separately (Rai, J. *et al.*, in preparation).

**Concentration of Antigens by Dialysis against Sucrose.**—The antigen to be concentrated was placed in a dialysis bag. Both ends of the bag were tied and the bag was kept in a beaker or a measuring cylinder. Solid sucrose covered the dialysis bag all around and the container was kept at room

temperature or at 4° C. After 8 to 24 hours the antigen was collected from the dialysis bag and processed further to evaluate its potentialities for routine serological tests. The proportion of solid sucrose to fluid containing antigen is not critical, but should cover completely the dialysis bag in the container. Whenever saturated sucrose solution was used, it was kept in a bottle or beaker containing a magnet and the dialysis bag was immersed in the solution which was stirred with magnet. The proportion of saturated sucrose solution was ten times more by volume to fluid containing antigen in the dialysis bag.

An alternative procedure to the above method was also tried and found to be satisfactory. LKB filter frame was inserted into an inflated dialysis bag and solid sucrose was placed in it. The dialysis bag was then immersed in a beaker containing antigen and kept for concentration. After 24 hours antigen concentrate was collected from the beaker.

The concentrated tissue culture antigen was compared with SA extracted mouse brain antigen in HI and CF tests employing hyperimmune mouse sera/peritoneal fluid against JBE, West Nile, dengue-1, dengue-2, dengue-3 and dengue-4 viruses. A similar comparative study was carried out with limited number of convalescent human sera/survey sera collected from different epidemics and surveys.

#### RESULTS AND DISCUSSION

About 10 fold increase of HA and CF titres of the antigen was achieved by dialysing the antigens against sucrose (Table I). The results of comparative study with the two antigens (SA extracted mouse brain antigen and Vero tissue culture sucrose concentrated antigen) showed no significant

TABLE I  
HA and CF titres of JBE antigens before and after concentration\*

Antigen lot number	HA titres at pH 6.4-6.6		CF titres†	
	Before concentration	After concentration	Before concentration	After concentration
1	40	480	16	128
2	80	960	ND	ND
3	ND	ND	4	64

\* About ten-fold concentration in volume obtained.

† The highest dilution of antigen which fixed 2.5 units of complement in the presence of hyperimmune ascitic fluid. ND=Not done.

difference in the HI and CF titres of the convalescent and immune sera (VRC unpublished data, 1974). Therefore, it is suggested that the sucrose

concentrated JBE antigens can be used in serological tests.

One of the applications of dialysis method is the concentration of solutions of macromolecules. Virus concentration is carried out by utilising hygroscopic macromolecules surrounding the dialysis bag. Polyethylene Glycol (PEG) has been used successfully for the concentration of virus and viral antigens including Togaviruses (Klemperer and Pereira; Della-Porta and Westaway).<sup>13-6</sup> Viral antigens have also been concentrated by dialysis method using Carboxymethyl Cellulose (Versteeg)<sup>19</sup>.

It is relatively easy to produce large volumes of infected tissue culture fluid for many arboviruses, and if HA and CF antigens are present in low titre the method described by us will provide a valuable supplement to the more commonly used antigens prepared from suckling mice. The method is rapid, reliable, economical and no special equipment or expensive material is required. Under the experimental condition described above, about ten-fold concentration was achieved in 8-24 hours. No denaturation effects were observed in spite of sucrose being present in the antigen concentrate. After dehydrating the sucrose, the same material could be reused repeatedly and was found to be satisfactory. Saturated sucrose solution could also be reused. Comparative studies on different methods of concentration of virus antigens are in progress to evaluate the merits of the technique described in this communication.

#### CONCLUSION

Sucrose dialysis can be employed to obtain high titred JBE, HI and CF antigens prepared from Vero culture.

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## A MOLECULAR ORBITAL TREATMENT OF SOME HALOGEN SUBSTITUTED AMIDES

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### ABSTRACT

A Huckel molecular orbital treatment of some halogen substituted formamide, acetamide and benzamide series has been carried out to study the effect of substitutions (Cl, Br, I) on the charge delocalization in these molecules. The net charges on carbon, oxygen, nitrogen and halogen atoms and the bond orders of the C = O and C — N bonds have been calculated and the results are discussed. The barrier heights for the internal rotation about the C — N bond and resonance energies are also calculated.

### INTRODUCTION

It is well known that in amide molecules, no single valence bond structure is consistent with all their properties. This is due to the delocalization of the carbonyl  $\pi$ -electrons and lone pair electrons of nitrogen, resulting in the double bond character of the C — N bond<sup>1-5</sup>. Infrared, Raman and NMR Spectroscopic studies of halogen substituted amides have been carried out by Petterson<sup>6</sup>, Laches<sup>7</sup> and recently by Deklein<sup>8,9</sup> and Devia<sup>10</sup>. In the present work, the authors have attempted to make a systematic study of electronic charges on O, C, N and halogen atoms and bond orders of C = O and C — N groups to study the charge delocalization due to different substitutions.

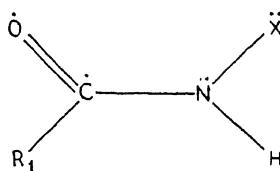
### METHOD OF CALCULATION, RESULTS AND DISCUSSION

The Huckel molecular secular determinant of a representative secondary amide is as shown in Fig. 1. Where the  $\alpha$  and  $\beta$  are the coulomb and resonance integrals respectively of the atoms O, C, N and the substituent to R<sub>1</sub> and X.

The secular determinant was solved using a CDC 3600 computer. The solution of the determinants provides the eigen values and eigen functions of the energy levels. Treating this as a six electron problem, the three lowest levels are taken as filled.

The mobile bond orders and the  $\pi$ -electronic charge defined in the usual way were determined. The net charge was determined as the difference between the charge the atoms would have in the absence of delocalization and its actual calculated electronic charge. A positive sign denotes a deficit

and a negative sign an excess of electronic charge. T. Yanezawa *et al.*<sup>11</sup> parameters are used in the calculations.



$\alpha_{O-E}$	$\beta_{C=O}$	0	0	0
$\beta_{C=O}$	$\alpha_{C-E}$	$\beta_{C-N}$	0	$\beta_{C-R_1}$
0	$\beta_{C-N}$	$\alpha_{N-E}$	$\beta_{C-X}$	0
0	0	$\beta_{C-X}$	$\alpha_{X-E}$	0
0	$\beta_{C-R_1}$	0	0	$\alpha_{R_1-E}$

Fig. 1

The results are tabulated in Tables I and II. As the aim of the present investigation is to study the relative delocalization of the charge due to the different halogen substitutions, the HMO method is used. This method is well suited for comparative study and the results depend upon the choice of the parameters of the coulomb and the resonance integrals,

In halogen substituted amides the net charge on oxygen increases from formamide to acetamide and acetamide to benzamide whereas the corresponding deficit in charge is decreasing on carbon atom. On the other hand the net charge on nitrogen atom shows an increase in the deficit of the charge. In formamide series the net charge on

benzamide series. The C-N bond order in formamide series shows a decrease from chloro to bromo to iodo. Similar variation is found in acetamide and benzamide series.

In Table II, the calculated bond orders of C=O bonds and corresponding stretching frequencies<sup>8</sup> are presented. The net charge on nitrogen atom

TABLE I

Net charges of oxygen, carbon, nitrogen and halogen atoms and bond orders of C=O and C-N bonds in halogen substituted amides

	Net Charges on				Bond Orders	
	Oxygen	Carbon	Nitrogen	Halogen	C-O	C-N
1. Chloro Formamide ..	-0.729	+0.344	+0.359	+0.026	1.603	1.695
Chloro Acetamide ..	-0.764	+0.242	+0.663	+0.118	1.519	1.693
Chloro Benzamide ..	-0.771	+0.238	+0.701	+0.134	1.516	1.685
2. Bromo Formamide ..	-0.728	+0.348	+0.353	+0.027	1.606	1.690
Bromo Acetamide ..	-0.765	+0.230	+0.654	+0.150	1.521	1.685
Bromo Benzamide ..	-0.772	+0.226	+0.690	+0.174	1.514	1.675
3. Iodo Formamide ..	-0.723	+0.365	+0.345	+0.013	1.612	1.683
Iodo Acetamide ..	-0.785	+0.242	+0.625	+0.148	1.530	1.683
Iodo Benzamide ..	-0.765	+0.237	+0.658	+0.286	1.522	1.675

oxygen is increasing as we go from Iodine to Bromine to Chlorine. But in acetamide series the net charge on oxygen is decreasing as we go from iodine to bromine to chlorine. Compared to chloro and bromo benzamide the iodobenzamide has less charge on oxygen, the variation of charge on oxygen being small.

The charge on nitrogen atom in all the three series (*i.e.*) formamide, acetamide and benzamide show less deficit as we go from Chloro to Bromo to Iodo substitutions. These results indicate that a relatively large delocalization is taking place from nitrogen lone pair electrons to carbonyl bond.

The bond orders of the C=O and C-N bonds as obtained by the authors in these studies are of the same order as obtained by Morris *et al.*<sup>1</sup> in case of primary amides like formamide and acetamide. The carbonyl bond orders in all halogen substituted amides show decreasing tendency from formamide to benzamide. The carbonyl bond order in formamide shows an increase from chloro to bromo to iodo substituted amides. Similar variations are observed in the case of acetamide and

and N-H stretching frequencies<sup>8</sup> are correlated. The more positive the nitrogen atom means, the lesser the strength of the N-H bond and hence a decrease in the N-H stretching frequency.

TABLE II

Frequencies and bond orders of carbonyl bond in halogen substituted acetamide and benzamide

Amide	$\nu(\text{C}=\text{O})$	Bond order	Charge on N atom	N-H stretching frequency
N-Cl Acetamide	1,728	1.519	+0.663	3,420
N-Cl Benzamide	1,714	1.516	+0.701	3,416
N-Br Acetamide	1,717	1.521	0.654	3,422
N-Br Benzamide	1,704	1.514	0.690	3,418

The barriers to internal rotation about C-N bond of these amides are calculated using the concept of *cis-trans* localization energy. In order to undergo *cis-trans* isomerization the system must

pass through a stage in which N-X  $\pi$ -electrons on one hand and carbonyl  $\pi$ -electrons on other hand are uncoupled. This stage may be taken as transition stage and the energy needed to bring the

pronounced in N-iodo amides compared to those of the corresponding N-chloroamides.

The resonance energies are calculated as the difference between the total energy of delocalized

TABLE III

*The pielectronic energy of N-halogen substituted amide and its fragments along with calculated barrier to internal rotation and C-N bond order*

Amide	Electronic energy	electronic energy of the fragments		Barrier to internal rotation in $\beta$	C-N bond order	Resonance energy in $\beta$
		Structure	Structure			
1. N-Chloroformamide	$6a+11.903\beta$	$2a+5.464\beta$	$4a+5.600\beta$	0.839	1.695	0.839
N-Chloroacetamide	$6a+12.260\beta$	$2a+5.616\beta$	$4a+5.600\beta$	1.044	1.683	1.196
N-Chlorobenzamide	$6a+12.287\beta$	$2a+5.621\beta$	$4a+5.600\beta$	1.066	1.685	1.223
2. N-Bromoformamide	$6a+11.094\beta$	$2a+5.464\beta$	$4a+4.800\beta$	0.830	1.690	0.830
N-Bromoacetamide	$6a+11.456\beta$	$2a+5.616\beta$	$4a+4.800\beta$	1.040	1.685	1.192
N-Bromobenzamide	$6a+11.486\beta$	$2a+5.621\beta$	$4a+4.800\beta$	1.065	1.675	1.222
3. N-Iodoformamide	$6a+10.681\beta$	$2a+5.464\beta$	$4a+4.400\beta$	0.817	1.683	0.817
N-Iodoacetamide	$6a+11.028\beta$	$2a+5.616\beta$	$4a+4.400\beta$	1.012	1.683	1.164
N-Iodobenzamide	$6a+11.056\beta$	$2a+5.621\beta$	$4a+4.400\beta$	1.035	1.675	1.192

amide system to this particular stage as a measure of barrier. Since uncoupled  $\pi$ -electrons remain conjugated to molecular fragment on its side, the representation of transition stage involves two radical fragments (structure II and III).

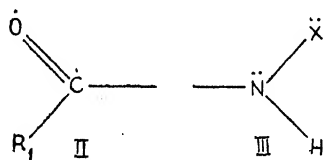


FIG. 2.

Thus the energy which must be spent to bring the molecule in this state is the difference between the energy of whole conjugated molecule and sum of the energies of fragments. This then is a measure of barrier to internal rotation.

The  $\pi$ -electronic energy of whole molecule, of fragments, barrier to internal rotation and C-N bond orders are presented in Table III. It is seen from the observations in Table III that the height of the potential is less in formamide series compared to that in the corresponding molecules of acetamide and benzamide series. The barrier to rotation decreases from N-chloro, to N-bromo and N-iodo amides in each series and this is very

pronounced in N-iodo amides compared to those of the corresponding N-chloroamides. The resonance energies are calculated as the difference between the total energy of delocalized

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## LETTERS TO THE EDITOR

A PLANE-SYMMETRIC UNIVERSE FILLED  
WITH PERFECT FLUID

THE geometry of the universe is described by the metric,

$$ds^2 = - \left\{ aT - \frac{4}{T} \right\}^2 dx^2 - T^2(dy^2 + dz^2) + dT^2, \quad (1)$$

where  $a$  is a positive arbitrary constant. From the field equations of the relativistic gravitation for a perfect fluid distribution,

$$R_j^i - \frac{1}{2} R g_j^i + \Lambda g_j^i = -8\pi \{ (p + \epsilon) V^i V_j - p g_j^i \}, \quad (2)$$

the pressure  $p$  and the density  $\epsilon$  of the distribution in the model are given by,

$$8\pi p = \Lambda - \frac{1}{T^2}, \quad (3)$$

$$8\pi \epsilon = \frac{3}{T^2} + \frac{16}{T^2(aT^2 - 4)} - \Lambda. \quad (4)$$

The physical conditions  $\epsilon > 3p > 0$  demand that the time  $T$  should be restricted by the inequality,

$$\frac{1}{T^2} < \Lambda < \frac{3}{2T^2} + \frac{4}{T^2(aT^2 - 4)}. \quad (5)$$

The coordinate system turns out to be comoving with  $V_4 = 1$ . The flow vector  $V_i$  satisfies the equations of the geodesics  $V^i_{;j} V^j = 0$  and hence the lines of flow are geodesics. From the study of the geodesic equations we conclude that a particle initially at rest in the model remains permanently at rest. The universe described above is irrotational but not shear free. The non-vanishing components of the shear tensor,  $\sigma_j^i$ , and the scalar of dilation,  $\theta$ , for the model are given by,

$$\sigma_{11} = \left\{ \frac{(6aT^2 - 8)(aT^2 - 4)}{3T^3} \right\},$$

$$\sigma_{22} = \sigma_{33} = \left\{ \frac{2T(3aT^2 - 8)}{3(aT^2 - 4)} \right\},$$

$$\theta = \left\{ \frac{(3aT^2 - 4)}{T(aT^2 - 4)} \right\}.$$

The model admits a four parameter group of motions. The non-vanishing components of the Weyl conformal curvature tensor,  $C_{hijk}$ , for the model are given by,

$$\begin{aligned} C_{12}^{12} &= C_{13}^{13} = C_{21}^{21} = C_{34}^{34} \\ &= -\frac{1}{2} C_{14}^{14} = -\frac{1}{2} C_{23}^{23} \\ &= \left\{ \frac{-8}{3T^2(aT^2 - 4)} \right\}, \end{aligned}$$

Petrov-Pirani classification of the model reveals that it is of Petrov type I D.

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## SIMULTANEOUS SPECTROGRAPHIC DETERMINATION OF VOLATILE AND REFRACTORY IMPURITIES IN TANTALUM OXIDE USING A D.C. ARC EXCITATION

SIMULTANEOUS determination of volatile and refractory impurities in tantalum oxide is generally done by a.c. arc method<sup>1,2</sup>. Since this source of excitation is not commonly available as compared to d.c. arc in smaller laboratories, a method for simultaneous determination of impurities using the conventional d.c. arc was developed. Methods for determination of either volatile<sup>3,5</sup> or refractory impurities<sup>6</sup> are available but no method is reported for simultaneous determination using a d.c. arc. Our experiments showed that using proper weight ratio of ZnO and graphite as buffer all impurities could be simultaneously determined. The method developed now estimates 12 impurity elements which include refractory elements like Nb and Zr and volatile elements like Pb and Sn in tantalum oxide.

Standards are prepared synthetically by dry-mixing the spec-pure grade oxides with tantalum oxide. All compounds used are supplied by Johnson Matthey and Co. 30 mg of a mixture of sample (standard), ZnO and graphite (1:1:1) is loaded in the crater of a 1" U.C.C. electrode. The sample as anode is excited at 15 amperes in a d.c. arc. The spectrum is photographed in the region 2300 Å to 3500 Å on Ilford N. 30 emulsion employing a JACO 3-4 metre grating spectrograph, in the first order of a 1200 grooves/mm grating blazed at 3300 Å.

Different weight ratios of sample, ZnO and graphite tried during experiments are 3:1:2, 2:1:1, 1:1:1 and 1:0:1. The ratio of 1:1:1 is found to give best overall sensitivity and uniform volatilisation of impurities.

A weak line of Ta at 2974.6 Å is selected as an internal standard. Other analytical details are given in Table I.

It is also found that tantalum oxide used for preparation of standards is impure and contains following elements: Fe—5 ppm, V—10 ppm, Al—15 ppm, Mo—20 ppm, Nb and Zr—30 ppm and Si—40 ppm. These elements are determined by the method of trial additions. The working curves

are drawn after correcting for these residuals. The working curves are found to be linear and close to 45° in inclination.

TABLE I

Analytical data for determination of impurities in tantalum oxide

Sl. No.	Analytical line (Å)	Determination Range (ppm)	Precision ± %
1.	Al 3092.7	17.5-1,000	23
2.	Fe 2966.9	10-2,000	28
3.	Pb 2833.1	5-200	18
4.	Mn 2576.1	2.5-1,000	22
5.	Mo 3112.1	60-4,000	22
6.	Ni 3423.7	20-2,000	19
7.	Nb 2950.8	130-4000	19
8.	Si 2524.1	50-1,000	25
9.	Sn 3034.1	10-2,000	24
10.	Ti 3186.0	50-2,000	21
11.	V 3118.3	30-2,000	18
12.	Zr 3391.9	40-1,000	11

The sensitivity reported here is lowest for Al and Sn.

The authors express their sincere thanks to Dr. N. A. Narasimham for his interest in the work. Our thanks are also due to Dr. S. L. N. G. Krishnamachari and Dr. V. B. Kartha for going through the manuscript and making valuable suggestions.

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# THE USE OF O-NITROPHENYLSULPHENYL GROUP FOR N-PROTECTION DURING THE SYNTHESIS OF THE PROTECTED HEXAPEPTIDE SEQUENCE (5-10) OF HUMAN FIBRINOPEPTIDE-A

THE human fibrinopeptide-A has the amino acid sequence. Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-  
1 2 3 4 5 6 7 8 9 10  
Glu-Gly-Gly-Gly-Val-Arg. The protected hexa-  
11 12 13 14 15 16  
peptide, Boc-Glu(OBzl)-Gly-Asp(OBzl)-Phe-Leu-Ala-OMe\* (1) corresponding to the sequence (5-10), has already been synthesised by us<sup>1</sup> using *t*-butoxy-carbonyl group for N-protection throughout. The present report concerns the synthesis of the hexapeptide fragment (5-10) using the O-nitrophenylsulphenyl (NPS) group throughout for N-protection. This was considered desirable as O-nitrophenylsulphenyl chloride required for the introduction of NPS group into amino acids is less expensive and easier to prepare.

The NPS-amino acids required for this investigation were prepared according to the procedure of Zervas<sup>2</sup> and with the exception of NPS-Gly, they were isolated as their dicyclohexylammonium salts. The NPS-amino acids were liberated from their salts at the time of condensation by treatment with 5% aqueous citric acid solution. During the synthesis dicyclohexylcarbodiimide was used for effecting condensation<sup>3</sup> and 2 N hydrogen chloride in ethyl acetate was used for the removal of NPS-group.

The condensation of NPS-Leu with Ala-OMe gave NPS-Leu-Ala-OMe as a gum (80%) which was deprotected to yield Leu-Ala-OMe.HCl (61%). This was reacted with NPS-Phe to afford NPS-Phe-Leu-Ala-OMe (50%), m.p. 90-91°, from which NPS group was removed to obtain Phe-Leu-Ala-OMe.HCl (96%), m.p. 180°. The neutralisation of this hydrochloride with 50% aqueous potassium carbonate furnished the free base, Phe-Leu-Ala-OMe, m.p. 196-199°, (Found: C, 62.76; H, 8.02; N, 11.32%. C<sub>19</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub> requires C, 62.79; H, 8.04; N, 11.56%). Further coupling of NPS-Asp(OBzl) with Phe-Leu-Ala-OMe led to NPS-Asp(OBzl)-Phe-Leu-Ala-OMe (65%), m.p. 181°, which was deprotected. The resulting hydrochloride (60%) on treatment with 50% aqueous potassium carbonate furnished Asp(OBzl)-Phe-Leu-Ala-OMe, m.p. 140-144° (Found: C, 63.27; H, 7.11; N, 9.83%. C<sub>30</sub>H<sub>40</sub>N<sub>4</sub>O<sub>7</sub> requires C, 63.36; H, 7.09; N, 9.85%). Condensation of this tetrapeptide with NPS-Gly gave NPS-Gly-Asp(OBzl)-Phe-Leu-Ala-OMe (74%), m.p. 191-192°. After deprotection of the pentapeptide, the resulting hydrochloride (90%) was reacted with NPS-Glu(OBzl) to yield NPS-Glu(OBzl)-



Gly-Asp(OBzl)-Phe-Leu-Ala-OMe (52%), m.p. 160–162°. Removal of NPS group from this hexapeptide resulted in Glu(OBzl)-Gly-Asp(OBzl)-Phe-Leu-Ala-OMe.HCl, m.p. 162–165°, identical with the product obtained from the deprotection of I. When the NPS group was used for N-protection the overall yield decreased considerably to 3.2% as against 23% when Boc-group was used.

We are thankful to Prof. M. V. Bhatt of the Indian Institute of Science, Bangalore, for the Micro-analyses.

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\* The amino acids used, with the exception of glycine, are of the L-variety. Abbreviations used are in accordance with the recommendations of IUPAC-IUB Commission on Biochemical Nomenclature.

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#### EMISSION SPECTRUM OF SnCl ( $C \rightarrow X^2\Pi_{1/2}$ ) SYSTEM

ALTHOUGH the absorption spectrum of SnCl ( $C \leftarrow X^2\Pi_{1/2}$ ) system was recorded as early as 1942 by Fowler<sup>1</sup>, there has been no report on the emis-

sion spectrum of this system, which formed the basis of the present investigation.

hence it becomes difficult to make out the bands clearly. A comparison of the bands in absorption and in emission recorded here are summarized in Table I.

TABLE I  
 $C \leftrightarrow X^2\Pi_{1/2}$  system

$v', v''$	Wavelengths of the Absorption Band Heads in Å (Fowler <sup>1</sup> )	Wavelengths of the Emission Band Heads in Å
1, 3	2323.80	2323.80
0, 2	2326.12	2326.12
0, 1	2307.42	2307.45
0, 0	2288.88	2288.89
1, 0	2268.62	2268.68
2, 0	2248.86	..

Even after several trials, it was not possible to observe the last (2, 0) band in emission.

However, we have recorded a number of new bands (about twenty-five) of the same system: the detailed analysis of which will be published shortly.

The authors acknowledge gratefully the award of the Junior Research Fellowship by U.G.C. to one of the authors (P. H. K.).

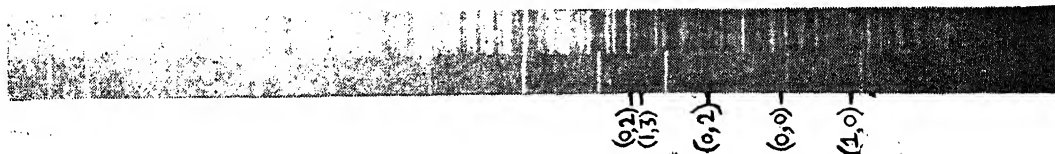


FIG. 1. Emission spectrum of SnCl ( $C \rightarrow X^2\Pi_{1/2}$  system).

sion spectrum of this system, which formed the basis of the present investigation.

The excitation source was a discharge tube to which the vapour of SnCl<sub>4</sub> (Analar Grade, B.D.H. make) was passed through a side arm via a needle valve. Bands were excited by a 15 K.V. (0.25 kw) Hilger transformer. The discharge was bluish-white in colour and tape-like in shape. The spectrum photographed by a Hilger medium quartz spectrograph is represented in Fig. 1. Careful observations (about twenty reproductions of the spectrum) revealed the presence of the emission bands (Table I).

The bands obtained are faint even after exposing the plate for a long time (15 hr). Further exposure increases the intensity of the background also and

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#### MANGANO-COBALTI-FERRITE MnCoFeO<sub>4</sub> SPINEL

*Preliminary information.*—The crystal structure of MnCoFeO<sub>4</sub> has been studied by Yagnik (1967) who has shown that it is a cubic spinel (Bragg, 1915; Nishikawa, 1915). Detailed structure is however not available. The present report concerns an independent determination. The charge distri-

bution of this compound has been determined by Yagnik (*loc. cit.*) by using Mossbauer spectroscopy. By using X-ray spectroscopy we have determined the ionic structure as  $Mn^{+2}$  ( $Co^{+3}$   $Fe^{+3}$ ) $O_4$ .

occupy the tetrahedral site similar to  $MnFe_2O_4$  spinel.

Comments.—Calculated lattice parameter from Mikheev's (1955) empirical relation does not agree

TABLE I  
Results of X-ray diffraction study of  $MnCoFeO_4$  spinel

$d$ in Å (obs.)	$d$ in Å (cal.)	I (obs.)	I* (cal.)	I† (cal.)	Plane $h$ $k$ $l$
4.861	4.853	15	16.6	16.3	1 1 1
2.978	2.972	30	33.2	33.7	2 2 0
2.527	2.534	100	100.0	100.0	3 1 1
2.096	2.101	30	26.4	31.4	4 0 0
1.717	1.716	15	11.4	12.5	4 2 2
1.619	1.618	40	38.4	39.6	5 1 1 } 3 3 3 }
1.488	1.486	60	56.5	56.4	4 4 0
1.281	1.282	20	23.2	24.8	5 3 3
1.096	1.095	15	13.9	13.9	7 3 1 } 5 5 3 }
1.054	1.051	5	5.7	4.6	8 0 0
0.9709	0.9707	3	2.0	2.0	5 5 5 } 7 5 1 }
0.8574	0.8580	3	2.0	2.0	8 4 4

Lattice Parameter  $a = 8.407$  Å.

Oxygen ion parameter  $\mu = 0.385$ .

\* By assuming the  $Mn^{+2}$  ions at the tetrahedral sites.

† By assuming the  $Fe^{+3}$  ions at the tetrahedral sites.

Crystal data.—(From Debye-Scherrer photographs, Fe  $K_{\alpha}$ ,  $\lambda = 1.93737$  Å)  $a = 8.407$  Å,  $Z = 8$ , space group:  $Fd3m$ , oxygen ion parameter  $\mu = 0.385$ .

Intensity data and structure determination.—All the observed reflections were indexed. The formation was taken to be complete when no lines of the reacting oxides were seen. The observed and calculated inter-planar distances ( $d$ ) and intensities of various reflection are included in Table I. In order to find out the site distribution the intensities are calculated for two models by assuming that the tetrahedral sites are either occupied by  $Fe^{+3}$  ions or  $Mn^{+2}$  ions. As both the ions  $Fe$  and  $Mn^{+2}$  have nearly same atomic scattering powers, the intensity data is indecisive. Hence the site distribution was determined from site preference energies (McClure, 1957; Dunitz *et al.*, 1957; Miller, 1959) of  $Mn^{+2}$  and  $Fe^{+3}$  ions and from the oxygen ion parameter. Both have shown that the  $Mn^{+2}$  ions

with the observed lattice constant which may be due to the differences in ionic radii.

The author is indebted to Prof. C. Mande for helpful suggestions and discussion.

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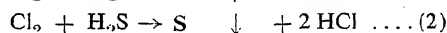
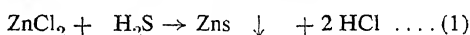
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# ELIMINATION OF DISSOLVED HYDROGEN SULPHIDE FROM BARIUM CHLORIDE SOLUTION

IN the manufacture of Barium Chloride from Barytes large amount of Hydrogen Sulphide is evolved and a certain amount of this Hydrogen Sulphide is absorbed by the mother liquor. The maximum amount of Hydrogen Sulphide thus absorbed may vary between 0.2 to 0.3 g/litre. When this mother liquor is concentrated to obtain Barium Chloride Crystals, this dissolved Hydrogen Sulphide is sufficient enough to corrode the evaporator tubes and the evaporator body. The Hydrogen Sulphide corrosion is prevented, at present, in the industry by heating this solution in open concrete tanks and expelling the Hydrogen Sulphide vapours using steam coils. Then the Hydrogen Sulphide free solution is evaporated in the evaporators. The use of steam to expel the undesirable Hydrogen Sulphide and the corrosion of mild steel steam pipes add to the cost of Barium Chloride.

the Sulphide was eliminated only to the extent of 50%. The volume of the solution is increased which increases the evaporation cost. The Bleaching powder<sup>2,3</sup> although removes the Hydrogen Sulphide to an extent of 85%, nearly 9 times the stoichiometric amount is needed to achieve this. Because of this excessive addition dissolved salt content increases three fold and there is considerable Barium loss. Hence addition of Bleaching powder was also found to be uneconomical.

Zinc Chloride<sup>3</sup> and Chlorine react with Hydrogen Sulphide quantitatively giving valuable by-products. The elimination of Sulphide is total in both the cases. The reactions are:



The Hydrogen Chloride thus liberated increases the acidity of the resulting solution which may be neutralised by adding Barium Hydroxide with further production of Barium Chloride. Thus Hydro-

TABLE I  
Effect of addition agents to remove Hydrogen Sulphide from the process liquor

No.	Additive Agent	Volume of Solution ml	pH	Amount Actually Added gm	Sulphide content			Barium Content			Ca and other salts as SO <sub>4</sub> <sup>++</sup>			% Contamination
					Initial gm	Final gm	% H <sub>2</sub> S removed	Initial gm	Final gm	% Loss	Initial gm	Final gm		
1.	Zinc Chloride (Sp. gr: 2.9)	200	4.5	1.5	0.0665	0.0008	99	40.03	40.0	..	0.74	0.742	..	
2.	Chlorine Gas	200	5.5	0.129	0.0258	..	100	39.7	39.7	..	0.56	0.56	..	

Hence it is desirable to evolve a process to remove Hydrogen Sulphide without using steam and with minimum corrosion losses.

An alternate method to remove this Hydrogen Sulphide is to add an addition agent which reacts with Hydrogen Sulphide without contaminating the product, Barium Chloride. When the reaction takes place the acidity is neutralised and in some cases the precipitated Sulphur may also be obtained as a by-product. The main criterion on which the addition agents are to be selected are: (i) neutralisation of acidity, (ii) recovery of Sulphur (iii) non-interference in the yield of the product Barium Chloride, (iv) minimum Barium loss, (v) elimination of heating as it increases the cost and the rate of corrosion.

Attempts were made to achieve this objective by using the following addition agents, (i) Spent Soap lye, (ii) Bleaching powder, (iii) Zinc Chloride solution, (iv) Chlorine gas.

The spent Soap lye<sup>1</sup> was found to be uneconomical as there was considerable Barium loss and

gen Sulphide is removed completely and the Barium loss is negligible. The percentage contamination by way of dissolved salts is also negligible. The results are shown in Table I.

It is recommended that either of the two additive agents, viz., Zinc Chloride or Chlorine can be added to eliminate the corrosion problem involved in the concentration of Barium Chloride solution due to the dissolved Hydrogen Sulphide.

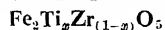
The authors wish to thank The Principal, Regional Engineering College, Tiruchirapalli-15, for his kind encouragement.

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## PHASE STUDIES IN THE SYSTEM



VARIOUS workers<sup>1-4</sup> studied the natural and synthetic samples of Pseudobrookite using X-ray diffraction techniques, but the present system is not

$c = 9.771 \text{ \AA}$ . The X-ray data of this new phase is given in Table II.

It is observed that the Pseudozirkelite and Pseudobrookite are isostructural. In all the mentioned compositions each unit cell contains four

TABLE I

*The system  $\text{Fe}_2\text{Ti}_x\text{Zr}_{(1-x)}\text{O}_5$*

Sample No.	Composition	Molecular weight	Colour	Density	Phase present
1.	$\text{Fe}_2\text{Ti}_{0.8}\text{Zr}_{0.2}\text{O}_5$	248.16	Brown	5.08	Pseudobrookite + Pseudozirkelite $a = 3.639 \text{ \AA}$ , $b = 9.509 \text{ \AA}$ , $c = 9.711 \text{ \AA}$ .
2.	$\text{Fe}_2\text{Ti}_{0.6}\text{Zr}_{0.4}\text{O}_5$	256.82	Deep brown	5.12	Pseudobrookite + Pseudozirkelite $a = 3.689 \text{ \AA}$ , $b = 9.536 \text{ \AA}$ , $c = 9.751 \text{ \AA}$ .
3.	$\text{Fe}_2\text{Ti}_{0.4}\text{Zr}_{0.6}\text{O}_5$	265.49	Deep brown	5.29	Pseudozirkelite $a = 3.706 \text{ \AA}$ , $b = 9.566 \text{ \AA}$ , $c = 9.771 \text{ \AA}$ .
4.	$\text{Fe}_2\text{Ti}_{0.2}\text{Zr}_{0.8}\text{O}_5$	274.15	Blackish brown	5.43	Pseudozirkelite $a = 3.706 \text{ \AA}$ , $b = 9.566 \text{ \AA}$ , $c = 9.771 \text{ \AA}$ .
5.	$\text{Fe}_2\text{ZrO}_5$	282.82	Pinkish brown	5.52	Pseudozirkelite $a = 3.706 \text{ \AA}$ , $b = 9.566 \text{ \AA}$ , $c = 9.771 \text{ \AA}$ .

reported in the literature. Hence in order to investigate the structural correlation of the compound  $\text{Fe}_2\text{ZrO}_5$  with Pseudobrookite and to observe the accommodating capacity of Pseudobrookite lattice for  $\text{Zr}^{4+}$  ions, this system is studied.

All the samples were prepared by mixing the appropriate quantities of iron (II) oxalate, Titanium dioxide and zirconium dioxide, followed by the usual ceramic techniques. The samples were fired at  $1050^\circ\text{C}$  for 24 hours and annealed to room temperature in air. The powders of these samples were analysed by a standard Philips diffractometer using  $\text{Mo K}\alpha$  radiation filtered through a Zr foil. The patterns obtained were used to calculate the lattice parameters and to observe the developed phases.

The results are given in Table I. From the ionic size considerations it is expected that on introduction of bigger ions like  $\text{Zr}^{4+}$  substituting the smaller ions like  $\text{Ti}^{4+}$ , should not form substitutional solid solution series without disturbing the lattice. However, in the present system it is observed that a series of substitutional solid solutions resulted with the swelling of the lattice. On introduction of even 20%  $\text{Zr}^{4+}$  ions into the lattice a new phase developed and predominated up to the last composition from the 60% concentration of  $\text{Zr}^{4+}$  ions in the lattice. This new phase is named for the first time as Pseudozirkelite and the pattern was indexed on the basis of orthorhombic symmetry with  $a = 3.706 \text{ \AA}$ ,  $b = 9.566 \text{ \AA}$ ,

formula units. The structure of these compounds is such that  $\text{Zr}^{4+}$ ,  $\text{Ti}^{4+}$  ions are octahedrally surrounded by six oxygen ions and the  $\text{Fe}^{3+}$  ions are

TABLE II  
*X-ray data of  $\text{Fe}_2\text{ZrO}_5$* 

$h/l_0$	$d$ in $\text{\AA}$	$hkl$
16	4.336	020
10	3.371	022
50	3.139	111
100	2.686	023
10	2.349	040
10	2.336	113
15	2.191	024
10	2.164	042
10	1.931	043
85	1.837	200
65	1.695	134
10	1.629	006
15	1.597	060
10	1.562	061
10	1.539	026
10	1.511	223
15	1.056	067

terahedrally surrounded by four oxygen ions. The combination of these polyhedra results in an orthorhombic unit cell.

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### A HIGH ALKALOID CONTAINING RACE OF *SOLANUM INCANUM* LINN., COLLECTED FROM THE PANIYAS OF KERALA

THE importance of the genus *Solanum* as a source of intermediates in the synthesis of cortisone has resulted in a search for high alkaloid containing Indian species of this genus. Amongst these *Solanum khasianum* with 5% and *Solanum elaeagnifolium* with 3% glyco-alkaloid have been reported by Maiti *et al.* (1964)<sup>1</sup> and *Solanum trilobatum* with 3.5% by Viswanathan (1973)<sup>2</sup>.

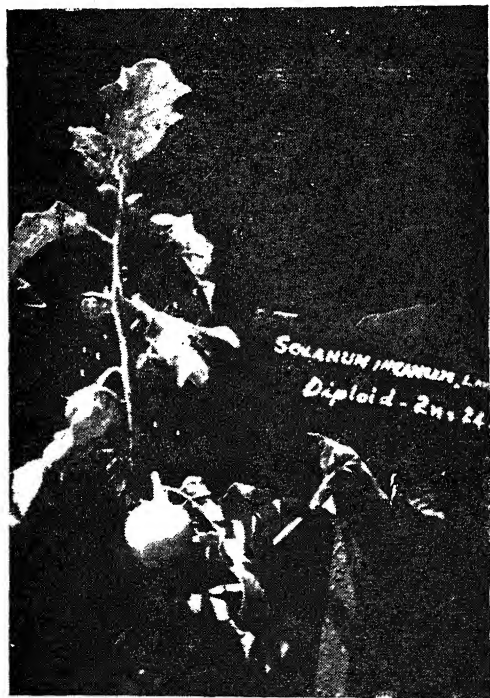


FIG. 1. A *Solanum incanum* Linn. plant from the Paniyas of Kerala with white fruits. Chro. No.  $2n = 24$ .

*Solanum incanum* Linn., a species closely related to *Solanum melongena*, Linn., the brinjal, with which it hybridizes easily is found wild throughout India. Several geographical races showing morphological differences exist in this species. Zutshi (1968)<sup>3</sup> reported 1.85% Solasodine in the Jammu variety and 2.2% in a race collected by the senior author from Kondotti, South Kerala. We collected 4 races from Kerala of which one was derived from a single fruit given by Valli Moopan, the Paniya Headman of Iritty, North Kerala, who said it was used as an oral contraceptive by the women of his tribe. The Paniyas are an aboriginal Tribe who lived on roots collected from the forest. They were famous for tiger hunting by spears as some of the African Tribes with whom they show some affinity.

Seeds of this "Paniya" variety of *Solanum incanum* were grown at the Ethno-botanical garden of the Field Research Laboratory, University of Madras. A uniform population of plants with white fruits was obtained. This character has not so far been observed in any variety of *Solanum incanum* collected by us, all of which had green fruits.

On chemical analysis of well-matured ripening fruits, this variety was found to contain 3.8 to 4.81% glyco-alkaloid which is the highest record for this species of *Solanum*.

*Solanum incanum* is as easy to cultivate as the brinjal. It yields 60–70 fruits per plant and it can easily replace *Solanum khasianum* as a source of glyco-alkaloid for commercial growing.

Centre for Advanced F. K. JANAKI AMMAL.  
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### A MEGA-PORPHYRITIC DOLERITE INTRUSION AT SAN PEDRO, GOA

THIS note describes an occurrence of a megaporphyritic dolerite intrusion at San Pedro in a road metal quarry on the south bank of the Mandovi river near Km 7/2 on the Panjim-Ponda road. Micaceous quartzite is the dominant rock type along the northern coast of Goa. These unfossiliferous pre-Cambrian metasediments of Cuddapah age (Oertel, 1958) are profusely intruded by basic dykes and are covered by a laterite capping of

variable thickness. The megaporphyritic dolerite body is an inverted saucer-shaped intrusion and the overlying quartzites show doming and fracturing hence referred to as laccolith. The contacts are sharp and do not show much baking effects. Large phenocrysts are irregularly and sparsely strewn in the body of the intrusion. Similar unusual type of basalt containing very large phenocrysts of plagioclase has been reported by Agashe and Gupte (1968) in the Deccan Trap basalts and also by Sowani and Peshwa (1964), from Purandar hills.

#### PETROGRAPHY

The rock consists of medium grained, greyish black doleritic groundmass with plagioclase phenocrysts. Many of the phenocrysts are exceptionally large (40 to 50 mm) and show a tendency to form glomeroporphyritic aggregates and make the rock a good example of porphyritic dolerite. The phenocrysts vary considerably in habit and size and in some cases the crystal faces are irregular. Some of the phenocrysts have a dusky brown interior and occasionally show criss-crossing of the groundmass within the crystals.

In thin sections the medium to coarse grained groundmass varies in texture from subophitic to doleritic. It is composed of plagioclase and ferromagnesian minerals in approximately equal amounts (Table I) or with an excess of feldspar. Groundmass plagioclase laths are randomly oriented and exhibit albite, carlsbad, albite-carlsbad and pericline twinning. They are altered to aggregates of calcite, epidote, clino-zoisite or zoisite and scattered flecks of white mica (sericite?). Pyroxenes (augite)

purplish brown in colour show faint pleochroism and have distinct cleavages. The colour suggests that it holds some titanium. Twinning with (100) as the twin plane is observed but it is not common. The pyroxene is affected by deuteritic or hydrothermal alteration and altered in varying amounts to fibrous light coloured bluish green amphibole (uralite). Some hornblende and biotite have been decomposed to form aggregates of fine grained magnetite. Twinning in the plagioclase megaphenocrysts is not clear due to deuteritic or later weathering alteration. Fresh parts of the crystals are colourless while altered portions show pale brown colour with greenish tinge. The crystal boundaries against the groundmass are generally irregular but in some crystals one or two margins remain straight. Groundmass has also crystallized in embayments and in cracks in the crystals. The occurrence of orange-tinged, thin cracks in the mineral, suggests iron migration perhaps due to weathering. Plagioclase phenocrysts are comparatively altered more than those in groundmass but the alteration product is the same. Quartz grains are found interstitial between feldspars and pyroxenes. Iron ore is seen throughout the sections as square, triangular, lath-shaped or irregular skeletal crystals and sometimes is enclosed in late minerals such as hornblende and biotite. Apatite needles are present in plagioclase and near the biotite-magnetite association.

The phenocrysts show a tendency to form glomeroporphyritic aggregates and most of them have corroded borders indicating their intratelluric formation and resorption during the process of

TABLE I  
Percentage by volume of the constituents of the groundmass (exclusive of the phenocrysts)

Sample No.	Plagioclase	Pyroxene	Uralite	Opaque minerals	Biotite	Quartz
SK-1	51.67	6.68	25.44	12.52	2.03	1.67
SK-2	53.86	13.73	22.04	6.17	1.32	2.88
SK-3	54.32	12.49	24.33	5.45	1.96	1.45
SK-5	50.62	20.73	15.43	8.26	2.68	2.27
SK-6	54.33	10.22	24.92	5.42	2.32	2.79
SK-8	51.21	25.32	11.09	8.68	1.21	2.49
SK-9	38.67	39.99	7.07	9.79	1.79	2.72
SK-10	48.32	29.92	7.90	9.34	3.24	1.26
SK-17	58.11	9.60	20.26	7.77	1.83	2.44
SK-18	55.78	17.75	17.83	7.04	0.61	0.99

eruption (Crookshank, 1936; Prasad, 1959). The presence of unusually large plagioclase phenocrysts poses certain problems regarding their history of formation. The mega-phenocrysts are early formed crystals of uniform composition characteristic of crystallization in slow cooling magma at depth. Their presence in a rock of doleritic texture shows that the magma in which they were included moved to higher levels where crystallization was more rapid.

The authors are indebted to Dr. N. K. Panikkar, the then Director, National Institute of Oceanography, for his interest in the work. They are also grateful to Shri H. N. Siddiquie and Shri R. R. Nair for going through the manuscript. One of us (B. G. Wagle) carried out the work on a CSIR Research Fellowship.

National Institute of

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#### PHENOXETOL A AS GOOD SORTING MEDIUM-CUM-PRESERVATIVE FOR ZOOPLANKTON IN THE TROPICS

ZOOPLANKTON samples collected during the International Indian Ocean Expedition (1960-65) and stored in the Indian Ocean Biological Centre were found to deteriorate progressively. Results of an analysis<sup>1</sup> of causative factors showed that bacterial action of the specimens partly caused by the sorting procedure may also be a cause. Hence of the need to find a sorting medium-cum-preservative (as part of the SCOR/UNESCO/WG-23 programmes) for the proper fixation and preservation of zooplankton.

Berry<sup>2</sup> reported the bacteriostatic and bactericidal value of ethylene glycol monophenyl ether. Rankin<sup>3,4</sup> used phenoxetol for treating fin rot in fishes. This was found active against penicillin resistant gram negative organisms. Use of propylene phenoxetol as a relaxing agent, as a preservative for a variety of animals and for preserving aqueous stains has been reported (Owen<sup>5</sup>; Owen and Steedman<sup>6,7</sup> and Turner<sup>8</sup>). Bagenal<sup>9</sup> and Rosewater<sup>10</sup> used propylene phenoxetol as an anaesthetic agent for fishes and molluscs.

Glycol monophenyl ether being referred to as phenoxetol is a colourless viscous liquid with a pleasant smell, soluble in water to about 2.5% v/v. This solution is perfectly stable with the pH around 5.5 to 6.0. Since it has no ionising groups, the pH of the water in which it is dissolved is unchanged and it has no buffering action of its own. A raised pH, however, can be obtained by adding the usual buffering agents. Though sea water has no chemical effect on phenoxetol owing to the salting out effect, distilled water was used for dilution. Normally phenoxetol is added to boiling distilled water, shaken until dissolved, cooled and adjusted to volume.

Initially 0.5% phenoxetol (B-phenoxyethyl alcohol or ethylene glycol monophenylether) or 0.5% propylene phenoxetol (3-phenoxypropanol) was tested as a sorting medium for subsampling and open dish sorting of zooplankton. A few drops of B.D.H. universal indicator whose pH range extended from 3 to 11 was also added to indicate the pH of the sorting medium. In general this was not found to have any damaging effect on the sorted plankton. During sorting more and more preservative was added to nullify the effect of evaporation. Examination of condition of these sorted taxa revealed no deterioration.

Being satisfied with its merit as a sorting medium, its use as a preservative was then tested. Zooplankton, fixed in 2% sea water-formaldehyde for ten days, was drained and after a quick rinse in distilled water, was transferred to a test series of different media. The two chemicals in different concentrations in different diluents were used (Table 1). The table also gives the various buffers used for neutralising the medium. Different additives for improving the condition of the specimens were also added to each of the preservatives in the experimental series.

The condition of the specimens were recorded for the last four years with emphasis on morphological features of taxonomic importance. The results indicate that distilled water solution was better than tap water and sea water. All four strengths of solutions used gave equally good results. However, 0.5% solution may be considered advantageous for proper preservation since it requires less quantity of chemical. The preservation was satisfactory in that retention of colour and pigmentation was good; specimens remained soft and flexible. Incidence of transparency of cuticular forms indicating a decay of tissue was negligible. Gelatinous plankters had their morphological features mostly retained. Calcareous plankton was well preserved in 3-5% potassium

TABLE I  
Test preservative used

Chemicals	Phenoxetol											
	Distilled water				Tap water				Sea water			
Strength % v/v	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
Condition* of organisms after four years	S	S	S	S	A	A	A	A	A	N	N	N

Chemicals	Propylene phenoxetol											
	Distilled water				Tap water				Sea water			
Strength % v/v	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
Condition* of organisms after four years	S	S	S	S	A	A	A	A	A	N	N	N

Buffers used: Hexamine, Borax, Marble powder and potassium oxalate in strengths of 0.0, 1.0, 5.0 and 10% w/v

\* S = Satisfactory; A = Average; N = Not satisfactory.

oxalate buffered medium. Addition of 1% sodium tetraborate improved preservation of chitinous and gelatinous plankton. The quick transfer of zooplankton from formaldehyde to phenoxetol had no visible osmotic damage or adverse effect.

To conclude, 0.5% phenoxetol or propylene phenoxetol in distilled water buffered with 1% sodium tetraborate was found to be an efficient sorting medium-cum-preservative after adequate fixation in 2% formaldehyde as recommended by Balachandran<sup>11</sup>. However the heterogeneous nature of the zooplankton makes it difficult to arrive at a single preservative solution equally good for all the taxa.

I gratefully acknowledge the assistance of Dr. N. K. Panikkar, former Director, National Institute of Oceanography, who suggested this investigation and Drs. M. Krishnan Kutty and M. J. George, Scientists, Regional Centre, National Institute of Oceanography, Cochin, for critically reading through the manuscript.

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## TWO NEW LEAF SPOT DISEASES OF CITRUS

DURING the investigation of plant diseases in Madhya Pradesh two new leaf spot diseases of *Citrus* were recorded. Microscopic examination of the infection spots revealed the presence of two different Sphaeropsidaceous fungus, *Bartalinia robillardoides* Tassi and a new variety of *Sphaeropsis tumefasciens* Hedges. Both these diseases have not been recorded previously from India. A brief description of the symptoms and morphology of the pathogen is given here.

The specimens have been deposited in the Herbarium Commonwealth Mycological Institute, Kew, England, and indicated in the text as I.M.I. number.

### 1. *Bartalinia* Leaf Spot of *Citrus medica* L.

Necrosis usually starts from apex downwards or at times from margin inwards. The mature spots are whitish to dirty on the upper leaf surface. High humidity favours the growth of the fungus (Fig. 1).

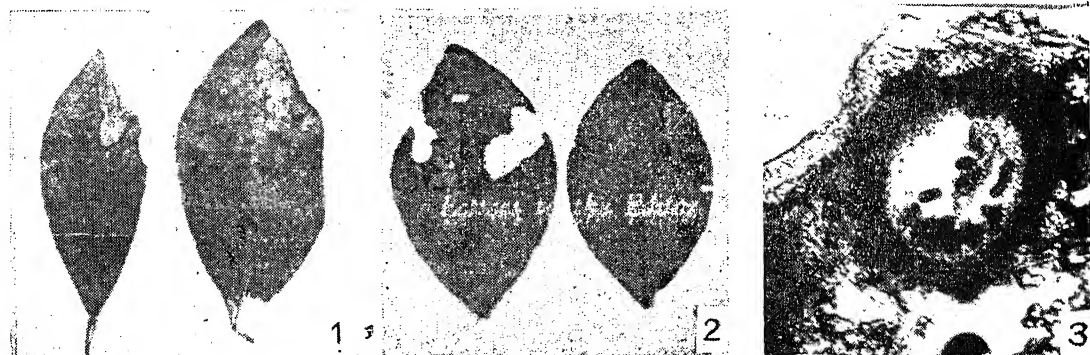
Pycnidia light brown, sub-epidermal, innate, depressed globose, ostiolate, 120–200  $\mu$  in diam.; conidiophores small, hyaline, slightly cylindric, 5.5–8  $\times$  3–4  $\mu$ ; conidia golden yellow, 4-septate, cylindric, 24–32  $\times$  4–5.5  $\mu$ ; apical cell conic, hyaline, bifurcate near the apex, apical appendages 12–24  $\mu$ , basal cell truncate, basal appendage lateral, exogenous, 4–16  $\mu$  long, 1.5  $\mu$  wide (I.M.I. Herb. No. 145462).



2. *Sphaeropsis* Leaf Spot of *Citrus decumana* L.

Disease usually starts from margin as amphigenous, ash-coloured spots and gradually increases downwards. Black puntiform pycnidia appear on both the sides but more on the upper side of the leaves.

agement and Mr. A. Johnston, Commonwealth Mycological Institute, Kew, for help rendered in the identification of species and to Principal, Government Science College, Jabalpur, for laboratory facilities.



Figs. 1-3. Fig. 1. Leaf Spot of *Citrus medica* L. Fig. 2. Leaf Spot of *Citrus decumana* L. Fig. 3. *Sphaeropsis tumefasciens* var. *citrum* Photomicrograph showing pycnidium and conidia.

Smaller veins are freely traversed but the larger veins act as a barrier to the spread of infection (Fig. 2).

Pycnidia dark brown, thick-walled, innate to erumpent, globose to subglobose, sub-epidermal, at times papillate, 90-230  $\mu$  in diam; conidiophores hyaline, small, simple; conidia large olive to dark brown or almost opaque, continuous, oblong-elliptical to cylindrical, episporium smooth, ends obtuse, 16-27  $\times$  10-12  $\mu$  (Fig. 3) (I.M.I. Herb. No. 143224).

In India *Sphaeropsis tumefasciens* Hedges has been reported from Ajitgarh (Rajasthan), by Prasad and Bhatnagar<sup>1</sup> as causing knots on branches of lime trees (*Citrus medica* var. *acida*). It has been reported by Sahni<sup>2</sup> causing a leaf spot disease on the same host. The present fungus has strictly single celled conidia (as against bicelled conidia in Prasad and Bhatnagar's) and wider than Sahni's material (5.1-8.5  $\mu$ ).

*Sphaeropsis tumefasciens* var. *citrum* var. nov.

Pycnidiiis atro-brunnea vel nigri crassiparietis innata vel erumpentia, globosa vel subglobosa, sub-epidermalis, nonnunquam papillata, 90-230  $\mu$  in diam; conidiophora hyalina, brevis, simplicia; conidiis magnus, olivacea vel atro-brunnea, unicellularia, oblongus-ellipticus vel cylindricus, episporio, laevi, extremo-obtusius, 16-27  $\times$  10-12  $\mu$ . Habit: Ad foliis viventibus *Citrus decumana* L. (Rutaceae), Agric. Univ. campus, Jabalpur, India, julio 1969. Leg. N. D. Sharma, typus positus in I.M.I., Kew, sub numero 143224.

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#### ON THE OCCURRENCE OF MYOGLOBIN IN THE NEPHRIDIUM OF THE GASTROPOD, *PILA VIRENS*

ALTHOUGH myoglobin has been reported in the radular, pharyngeal, stomach and heart muscles of gastropod molluscs<sup>1-3</sup> hardly any data are available on the occurrence of this heme pigment in their excretory organs. The present report deals with a histochemical and biochemical study of myoglobin in the nephridium of the freshwater prosobranch *Pila virens*.

For the histochemical study fresh frozen sections of 12 to 16  $\mu$  thickness, taken from nephridial tissues of anaesthetised animals, were processed according to the method of Chinoy<sup>4</sup>. The biochemical estimation was carried out spectrophotometrically, employing an adapted method of Tappan *et al.*<sup>5</sup>.

Of the two chambers constituting the nephridium in *Pila virens*, the posterior one showed a completely negative reaction whereas the anterior

chamber had significant amounts of myoglobin. The heme pigment was observed to be localised in the highly branched lamellae which project into the lumen of the chamber (Fig. 1). A significant gradation in the myoglobin content was also noticed, with the lamellae of the right side having markedly higher concentration than the left (Fig. 1). Biochemical assay revealed that the tissue of the anterior chamber had a myoglobin content of  $2.77 \pm 0.12$  mg/g wet weight. The posterior chamber did not have any estimable amount of myoglobin at all.

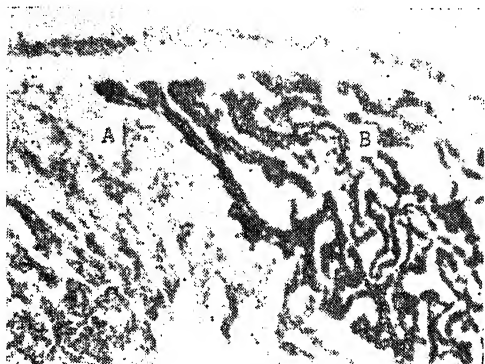


Fig. 1. C.S. of the anterior chamber of the nephridium of *Pila virens*. A and B. Lamellae showing gradation in myoglobin concentrations. Benzidine reaction,  $\times 280$ .

Apart from its classical role of functioning as an oxygen store in tissues, it has been suggested that myoglobin is capable of facilitating oxygen transport at a molecular level. Studies by Wittenberg<sup>6</sup> and Loizzi and Redmond<sup>7</sup> have clearly shown that myoglobin increases the availability of oxygen to tissues by increasing the rate of diffusion of it in a manner similar to that observed in hemoglobin films. This is particularly useful in tissues which are involved in repetitive activity. The present study revealed that myoglobin is selectively present in the lamellae of the anterior chamber of the nephridium of *Pila virens*. This is an original finding and to the best of our knowledge has not been reported for any other mollusc<sup>8</sup>. The investigations on the excretory system in *Pomacea*, a related genus of *Pila*, have shown that the anterior chamber is chiefly concerned with reabsorption and ionic regulation, and that its right side is more active than the left<sup>9</sup>. It is likely that the anterior chamber in *Pila virens* may also have a similar function, and possibly the presence of myoglobin in the lamellae may be of considerable help in facilitating the oxygen transfer necessary for such energy utilising functions. Recent observations on

tissue respiration in *Pila virens* have indicated that the anterior chamber has a comparatively higher oxygen consumption ( $4.08 \mu\text{l/mg/hr}$ ) than the posterior chamber ( $1.8 \mu\text{l/mg/hr}$ )<sup>10</sup>. This further supports the view that the anterior chamber is the more active portion of the nephridium in *Pila virens*.

We are indebted to Prof. K. K. Nayar for constant encouragement. Our thanks are also due to Mr. T. I. Jacob for technical assistance.

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#### SECRETORY BLEBS FROM THE SEMINAL VESICLES OF THE INDIAN FRUIT BAT, *ROUSETTUS LESCHENAULTI* (DESMAREST)

AN interesting feature of the secretion of the seminal vesicles of the Indian fruit bat, *Rousettus leschenaulti*, is that the secretion from each cell maintains its individuality and remains in the form of a spherical bleb for a considerable time. Hence, in sections of active seminal vesicles of this animal the lumen of the tubules are filled with a number of independent, nearly spherical droplets (Fig. 1), which do not run together to form a coagulum as in the case of the secretions from other glands. These blebs maintain their individuality even after they are ejaculated. Hence, the vagina is filled with these blebs for several days after copulation (Fig. 3).

The secretory material appears to come out of the cells along with an envelope of cytoplasm of the cell secreting it. Hence, the bleb has a covering of cytoplasm which gets pinched off from the cell after the bleb starts oozing out of the cell (Fig. 2). During the secretory phase of the seminal vesicle only one bleb emerges from the cell at a time, and the cell becomes considerably



FIGS. 1-3. Fig. 1. Section of the active seminal vesicle of *Rousettus leschenaulti* showing numerous independent secretory blebs within the tubules.  $\times 120$ . Fig. 2. Part of Fig. 1 magnified. Note the oozing out of a single bleb from each cell, and each bleb has a central darkly staining core with an envelope of lightly staining cytoplasm.  $\times 560$ . Fig. 3. Part of the section of the vagina of the female after copulation. Note the innumerable number of independent spherical blebs in the vaginal lumen.  $\times 60$ .

reduced in size after the bleb oozes out thereby indicating that a part of the cytoplasm of the cell gets lost with each bleb. Each bleb appears to have a dense core of secretion enclosed in cytoplasm of the cell secreting it. The central dense core of the bleb is strongly PAS-positive.

Detailed studies on the histochemistry of the seminal vesicle and the biochemistry of the secretion from this gland are in progress.

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Nagpur, February 28, 1974.

# SOME CHEMICAL CONSTITUENTS IN THE BLOOD PLASMA OF FOUR SPECIES OF FRESHWATER AIR-BREATHING FISHES

THE blood is more closely related with total metabolism of the animal and the composition of blood reflects the state of function at any time could be known by measuring the composition of the blood (Shell<sup>1</sup>).

Das<sup>2</sup> reported protein, lipoprotein, hemoglobin and glucose contents of the blood of three major carps of Bengal. Seasonal variations in the biochemical composition of blood serum of *Cirrhina mrigala* (Ham.) and *Labeo rohita* (Ham.) were investigated by Naseem and Siddiqui<sup>3</sup>. Khanna and Singh<sup>4</sup> reported the blood glucose level of *Channa punctatus* (Bloch).

the blood. To obtain plasma the blood was centrifuged at 3,500 rpm for 15 minutes. Standard methods described by Oser<sup>5</sup> were followed for the estimation of chemical constituents.

The amount of various chemical constituents in the plasma varies widely from species to species (Table 1). Total phosphorus values in different species studied ranged from 53.8 to 69.8 mg%, while the acid soluble phosphorus values from 25.2 to 41.3 mg%. *O. striatus* contained the highest percentage of cholesterol. Lipid phosphorus, inorganic phosphorus, glucose, iron, calcium and non-protein nitrogen values were higher in cat-fishes than those in murels. The quality of food consumed by the fish appears to affect the chemical constituents of the blood.

TABLE 1  
Chemical constituents of plasma of four air breathing fishes

Chemical constituents (mg%)	Cat-fishes		Murels	
	<i>C. batrachus</i> (22.0-25.0 cm) (30.0-170.5 gm) (15)	<i>H. fossilis</i> (20.0-24.0 cm) (75.0-190.0 gm) (14)	<i>O. punctatus</i> (90.5-22.0 cm) (105.0-135.0 gm) (15)	<i>O. striatus</i> (40-45 cm) (518-910 gm) (10)
Total phosphorus ..	63.8 ± 2.90	60.0 ± 1.67	53.8 ± 1.45	69.8 ± 1.00
Acid soluble phosphorus ..	32.2 ± 1.97	28.2 ± 0.57	25.2 ± 0.82	41.3 ± 0.76
Lipid phosphorus ..	21.5 ± 1.21	20.0 ± 0.73	16.1 ± 0.35	18.6 ± 0.80
Inorganic phosphorus ..	11.4 ± 0.28	11.8 ± 0.23	10.5 ± 0.42	9.7 ± 0.59
Glucose ..	60.5 ± 1.30	76.0 ± 1.91	50.0 ± 1.58	42.0 ± 0.99
Iron ..	44.6 ± 1.35	38.3 ± 1.08	29.0 ± 1.02	36.5 ± 0.75
Calcium ..	9.6 ± 0.31	10.0 ± 0.45	8.8 ± 0.50	8.9 ± 0.86
Cholesterol ..	432.0 ± 17.9	603.7 ± 14.5	546.0 ± 18.4	621.0 ± 26.9
Non-protein nitrogen ..	60.9 ± 1.83	65.5 ± 1.16	50.8 ± 0.72	62.9 ± 1.38

Length, weight and number of fishes in parenthesis. Mean ± S.E.

The amounts of total phosphorus, acid soluble phosphorus, lipid phosphorus, inorganic phosphorus, iron, calcium, cholesterol and non-protein nitrogen in the plasma and glucose in whole blood of *Clarias batrachus*, *Heteropneustes fossilis*, *Ophioccephalus punctatus* and *O. striatus* were estimated. The fishes were collected from the same pond and the analyses were completed within one month to avoid seasonal variations. Blood was collected after severing the caudal peduncle of each fish. For the analysis was used 3.2% sodium citrate solution for murels and double oxalate of ammonium and potassium for cat-fishes at the concentration of 4 mg/2 ml of the blood for checking the coagulation of

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# A NEW REPORT ON SECONDARY DORMANCY IN CERTAIN ARID ZONE SEEDS

*Merremia dissecta* Hallier, f. is a climber which appears to be very well adapted for hot and arid conditions of the western Rajasthan. Similarly, *M. aegyptia* (Linn.) Urban, is another arid zone species which grows in the rainy season and disappears with the departure of rain, although it has been reported to perennate by rootstock<sup>1</sup>. It has been shown that the age of the seed plays an important role in causing hard seed coatedness which disappears slowly with passage of time<sup>2</sup>, among arid zone members of Convolvulaceae; besides a few other families. Freshly harvested seeds were reported to give 4-6% germination in *M. dissecta*<sup>2</sup>. Seeds of three *Convolvulus* species occurring in arid areas were also reported to be impermeable to water<sup>3</sup>. Such seeds germinate only if their permeability is raised mechanically or chemically. However, these seeds became permeable

stage II to III and 4-6 days from stage III to IV, and seeds of stage V were completely mature. Except for the first two stages of maturity which may be due to after ripening period or undeveloped embryos, just freshly harvested seeds in both the species were able to germinate cent percent as is evident from Table I.

The hard seed coat dormancy which one detects in an old seed attached or detached from the mother plant, desiccated due to environmental conditions (dry air and high temperature) is a latter development. In other words, a very small percentage of seeds is able to germinate after the desiccation when the percentage of water inside the seed is 4.3 in *M. dissecta* and 2.0 in *M. aegyptia*. Experimentally it has been proved here that if the percentage of moisture in seeds reached such stages, beyond which germination was not possible, the result is a hard seed coat dormancy. The completely dry seeds showed a 100% germination after scarifica-

TABLE I

Percentage of seed moisture (24 h desiccation at 65° C) and germination (at the end of 3 days) in *M. dissecta* and *M. aegyptia* at different levels of seed maturity (I-V)

Plants	Percentage	Stages of seed maturity				
		I	II	III	IV	V (completely dry)
<i>M. dissecta</i>	Germination ..	0	0	100	100	5
	Moisture ..	60	55	47	42	4.3
<i>M. aegyptia</i>	Germination ..	0	5	100	100	5
	Moisture ..	65	57	55	33	2.0

gradually over a long period of time. Seeds of *M. aegyptia* have also been reported to show a hard seed coat dormancy<sup>4</sup>.

In the present studies on the ecophysiology of seed germination in these two species, it was observed that hard seed coatedness is not a primary nature of the seed dormancy. Seeds taken out from the mature fruits (but not completely dried); although showed certain morphological differences when compared with completely dried and old seeds, germinated upto hundred percent. Seeds of *M. dissecta* changed colours during different stages of maturity from just ivory white → brownish-white (with dark micropyle) → light brown → dark brownish-black. Successively, the seed size is reduced because of moisture loss on reaching complete maturity and dry state. In case of *M. aegyptia*, the seeds changed colours from white → greenish-white → greenish-yellow → lemon-yellow, during different stages of maturity. The stages of maturity, for both the species, took about 12-15 days from stage I to II; 8-10 days from

tions with conc. H<sub>2</sub>SO<sub>4</sub> acid (pretreatment) for 150 mts. and 50 mts. for *M. dissecta* and *M. aegyptia*, respectively. This is a fact which has remained hitherto undiscovered by many earlier workers in this field and is reported here. In nature, the desiccation of seeds is achieved slowly, spreading over a number of days, after which they present hard seed coat dormancy, a secondary development for which the structural differentiation has already taken place.

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# CYTOLOGICAL INSTABILITY OF WHEAT VARIETY KALYANSONA

INVESTIGATIONS were carried out to study the cytological stability of some semi-dwarf hexaploid wheat varieties, growing in the fields of the Indian Agricultural Research Institute, New Delhi, in the year 1972. The main objective of this study was to investigate the reasons involved in the decline of yield of wheat variety Kalyansona and its becoming susceptible to the rusts under field conditions.

and these gene(s) are hemizygous ineffective, then such plants would become susceptible to those races of rusts, the resistance of which is controlled by genes located on this univalent.

In this communication we would like to suggest that the cytological instability of Kalyansona may be one of the major factors responsible for the deterioration in its yielding ability and its becoming susceptible to the rusts. It is, therefore, desirable to breed varieties which are cytologically stable.

TABLE I

*Chromosome configurations per cell at metaphase I in these three varieties of wheat*

Variety	Cells scored	% of normal cells	I	III	IV	VI
Kalyansona	818	42	$105 \pm 01$	$08 \pm 01$	$35 \pm 02$	$002 \pm 001$
Nadadores mutant	686	92	$013 \pm 004$	$004 \pm 002$	$07 \pm 01$	$001 \pm 001$
Pb. C 591	622	91	$012 \pm 005$	$006 \pm 004$	$06 \pm 01$	..

Metaphase I of meiosis was studied in 25 plants each of varieties Kalyansona, Nadadores mutant and Pb. C 591, picked up at random from different plots.

1. The percentage of normal cells with 21 bivalents is only 42 in the case of Kalyansona whereas it is above 90% in the varieties Nadadores mutant and Pb. C 591. A hybrid-derived variety possessing about 90% normal cells is considered to be cytologically stable.

2. The high frequency of multivalents in Kalyansona (8% trivalents and 35% quadrivalents), which indicates translocations is expected to lead to deficiencies and duplications in both the male and the female gametes. The deficient gametes, when transmitted, could lead to the loss of resistance genes in Kalyansona within a few generations, if the chromosomes involved in interchanges possess resistance genes, with the result that a sizable part of the population would become susceptible.

Apart from these chromosomal aberrations observed in Kalyansona, it was found that out of 25 plants analysed cytologically, one monosomic plant ( $2n = 41$ ) was detected in this variety, which is an undesirable feature, as upon selfing monosomic plants would produce about 75% monosomics, apart from giving rise to nullisomics ( $2n = 40$ ), trisomics ( $2n = 43$ ) and tetrasomics ( $2n = 44$ ). Such an aneuploid population is also expected to bring down the yield of Kalyansona.

In addition to this limitation, if the univalent in the monosomic plant possesses resistance gene(s)

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## A NOTE ON THE FISH, *AMPHIPRION* *POLYMNUS* (LINN.), A NEW RECORD TO THE INDIAN COAST

IN the course of a survey on the seaweed resources of the intertidal coastline in Okha-Dwarka area, of the Arabian Sea, on 19th January, 1973, two fishes were found swimming close to a giant sea-anemone, *Stoichactis gigantea* (Forsk.) in a small puddle on the limestone reef off Mithapur ( $69^{\circ} 01' E$  Long.  $22^{\circ} 25' N$  Lat.). The fishes were identified as *Amphiprion polymnus* (L.). The specimens have been deposited in the museum of the Marine Biological Research Station, Port Okha.

*Amphiprion polymnus* distributed in the Indo-Australian Archipelago has been reported from Andamans to the Malay Archipelago and beyond (Day, 1878; De Beaufort, 1940). The present study extends the distribution of this species upto the Indian coast. Since earlier descriptions of the species were based on preserved specimens, a description of the live specimens is also included presently.

TABLE I  
Characters of various species of the Genus *Amphiprion*

Sr. No.	Characters	<i>A. sebae</i> (Day, De Beaufort, Smith, Munro) 3	<i>A. clarkii</i> (Day) <i>A. bicinctus</i> (De Beaufort, Smith) (Jones and Kumaran) 4	<i>A. chryso-gaster</i> (Jones and Kumaran) 5	<i>A. polymnus</i> (De Beaufort) 6	<i>A. bifasciatum</i> (Day) (= <i>A. polymnus</i> ) 7	<i>A. polymnus</i> (Present study) 8
1	D	X-XI, 14-15	X-16	X-16	X-XI, 13 (14)	XI, 13-15	X-15
2	A	II, 12-13	II, 13	II, 13	II, 12 (13)	II, 12-13	II, 12-13
3	P	2)	19	II, 17	2, 16-18	15	18
4	L. 1	34-40 (38)	38-40	44	35-41	..	38-40
5	L. tr	6/18	6-7/19	6/1/19	4-6/1/19-21	6/19	5/1/1
6	Sq. 1	50-55	55	..	5-55	50-55	52-55
7	No. of scales before dorsal	12-16	19-25	..	13-16	..	1-15
8	Fin	Increasing in length to the 6th, 4th equal to the length of head, 10th much shorter than 1st dorsal ray, soft dorsal higher than spinous dorsal	3rd and 4th dorsal spines longest, last spine shorter than 1st dorsal ray, soft dorsal rounded behind	..	2nd to 6th dorsal spines subequal, longer than others. Last spine much shorter than 1st dorsal ray. Soft dorsal rounded, much higher than spinous part	..	2nd to 6th dorsal spines subequal. Last spine shorter than 1st ray. Soft dorsal rounded
9	Anal	1st spine half length of 2nd, soft anal rounded as deep as soft dorsal	1st spine half length of 2nd, soft anal less deep than soft dorsal, acutely round behind	..	1st spine half length of 2nd, soft anal rounded, as deep as soft dorsal	..	1st anal spine half length of 2nd, soft anal rounded, as deep as soft dorsal
10	Pectorals	Rounded, equal to ventrals and to head without snout	Equal to or longer than rounded Pectorals	..	Pectorals and ventrals subequal	..	..
11	Ventrals	..	Enarginate, forked in large specimens	..	..	..	..
12	Caudal	Cut square or emarginate in adult	Brown	Dark brown	Slightly rounded	..	Slightly rounded
13	Colour	Dark brown	Brown	Dark brown	Dark brown or a most black	Brownish-black	Black (in living)
14		Pectorals, ventrals and anal deep brown or black. Caudal canary yellow	Snout, chest, pectorals and caudal yellow	Dorsal dark brown, last two spines, first two rays and distal parts of posterior rays yellowish-white, ventral yellowish-brown	Ventral anal dark brown the later, light in their distal part	..	Dorsal black except part covered by the second band
15		..	..	Anal brownish basally, lighter distally. Pectorals yellowish-brown	Caudal dark brown, edged with white	Caudal black with a white upper and lower edge	Ventrals and anal black
16		..	..	..	..	..	Pectorals black at base, white above

Band No.	Three	Three	Three	Three (Two)	Two	Two white, one yellow (Three)
Colour Position	Chalky or yellowish i. From nape, behind eye, over opercle	Chalky i. From nape, obliquely downwards along hind-border of eye, over posterior part of opercle to posterior part of inter and sub-opercle ii. Commencing at the base of the last five dorsal spines, passing to the front of the base of the anal fin iii. Covering the greater part of the caudal peduncle to the base of caudal	Yellowish i. From nape, obliquely downwards to opercle ii. From ninth dorsal spine and second or third dorsal ray to just before anal fin iii. Across caudal peduncle	Chalky white i. From nape, immediately behind eye, over pre-opercle and opercle to sub and interopercle ii. Beginning on posterior part of spinous dorsal and extending on interior part of soft dorsal or almost to its end, continued downwards to anus tapering here and joining that of the other side or not iii. The whole of the soft dorsal white and the band going not further downwards than middle of the sides iii. Covering the posterior half of the caudal peduncle	Milk white i. From nape, passing over the opercles, just touching the posterior edge of the orbit ii. From the last three dorsal spines and continuing downward to the middle of the body and backward to the summit of all the dorsal rays iii. Covering almost whole caudal peduncle	Two white, one yellow i. From nape, immediately behind eye over opercle ii. From the last three dorsal spines continuing downward to anus tapering here and joining that of the other side and backward to the summit of all the dorsal rays iii. Covering almost whole caudal peduncle
	Chalky or yellowish i. From nape, behind eye, over opercle	Chalky i. From nape, obliquely downwards along hind-border of eye, over posterior part of opercle to posterior part of inter and sub-opercle ii. Commencing at the base of the last five dorsal spines, passing to the front of the base of the anal fin iii. Covering the greater part of the caudal peduncle to the base of caudal	Yellowish i. From nape, obliquely downwards to opercle ii. From ninth dorsal spine and second or third dorsal ray to just before anal fin iii. Across caudal peduncle	Chalky white i. From nape, immediately behind eye, over pre-opercle and opercle to sub and interopercle ii. Beginning on posterior part of spinous dorsal and extending on interior part of soft dorsal or almost to its end, continued downwards to anus tapering here and joining that of the other side or not iii. The whole of the soft dorsal white and the band going not further downwards than middle of the sides iii. Covering the posterior half of the caudal peduncle	Milk white i. From nape, passing over the opercles, just touching the posterior edge of the orbit ii. From the last three dorsal spines and continuing downward to the middle of the body and backward to the summit of all the dorsal rays iii. Covering almost whole caudal peduncle	Two white, one yellow i. From nape, immediately behind eye over opercle ii. From the last three dorsal spines continuing downward to anus tapering here and joining that of the other side and backward to the summit of all the dorsal rays iii. Covering almost whole caudal peduncle

Caudal pale yellowish or white with dusky brown area in the middle of fin

Caudal black edged with white, the black colour therefore forming a triangular patch, with the base coinciding with the caudal fin



*Amphiprion polymnus* (Linn.)*Perca polymna* Linn. 1758.*Amphiprion bifasciatum* Bloch, Day, 1878.*Amphiprion polymnus* (L.) De Beaufort, 1940 ;  
Smith, 1949.

D.X. 15. A. II, 12-13. P. 18, V. I, 5. L. Lat. 38-40 with 55 in horizontal series to caudal. L. tr. 5.1/19. The depth of the body was 2.85 and head 3.63 in the total length. Head length and height were equal. Eye was equal to snout and 3.6 in the length of head. Mouth was oblique. Both jaws had a single row of conical teeth. Pre-orbital had 1-2 spines and the hind border of preopercle was feebly denticulated. Opercular bones were covered with scales. The scalation on the head extended upto half the diameter of the eye. Number of rows of scales before the dorsal was 14-15. The specimens measured 56 mm and 40 mm in total length.

The colour of living specimens was black. Three broad transverse bands, two chalky white and one (on caudal peduncle) yellow, were present. The first band, from nape, passed over the opercles just touching the posterior edge of orbit. The second band commencing at the base of last three dorsal spines, extended over the anterior and upper margins of the soft dorsal and passed down to the front of the anal fin tapering and joining that of the other side. The third band covered the caudal peduncle, also bordering the caudal fin. Pectorals were black at the base and white along the free edge with a broad median yellow band. Dorsal was black except the part covered by second transverse band. Ventrals and anal were black. Caudal fin was black, edged with white. The edge was wider towards the lobes, the black colour, therefore, formed a triangular patch, with the base coinciding with the yellow base of caudal fin. The black triangular patch was much more profound in the larger specimen. Snout and chest were yellow.

The specimens collected presently agree with descriptions of *A. polymnus* given by Day (1878), De Beaufort (1940), Smith (1949), etc., but differ from them all (Table I) in :

(i) black body colour, (ii) yellow median band in pectorals, (iii) yellow caudal peduncle and/or the third transverse band, (iv) yellow chest and snout. They resemble *A. clarkii* and *A. sebae* in (i) yellow snout, chest and caudal peduncle (c.f. De Beaufort, 1940), (ii) three chalky white transverse bands in preserved specimens, (iii) D. X, 15. P. 18, and (iv) 14-15 rows of scales before dorsal. *A. polymnus* (present specimens) also resemble *A. chrysogaster* Cuvier and Valenciennes in having three bands and yellow caudal peduncle (Jones

and Kumaran, 1964) but in latter all the three transverse bands are yellow, the anal and ventrals are yellowish and pectorals lack the white free edges. It appears that the characteristics of the various species of the genus *Amphiprion* are variable thus requiring a restudy and revision.

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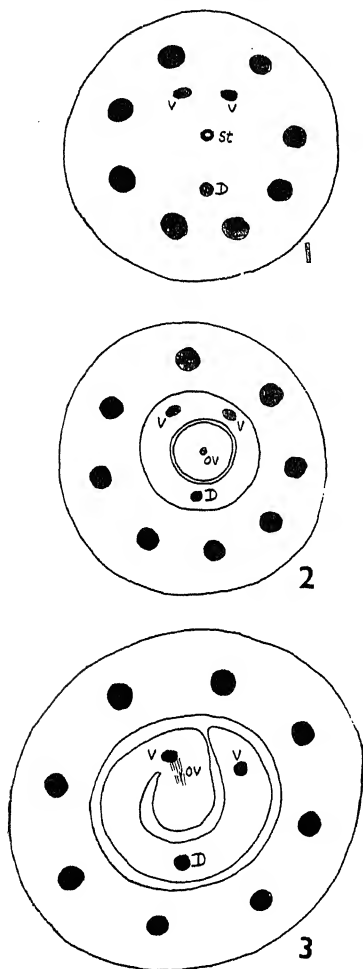
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#### THE PROBABLE ORIGIN OF THE BASAL PLACENTATION IN ELAEAGNACEAE

ELAEAGNACEAE is a small dicotyledonous family of three genera with a monocarpellary gynoecium and a single basal ovule. During investigations by the present author on the floral anatomy of this family, an interesting abnormal feature was observed in a few specimens, which could well represent the ancestral condition. The usual gynoecial organization and vascular supply to it in *Elaeagnus conferta* Roxb. with which the present account deals, is as follows. The ovary is monocarpellary, and the carpellary margins are only loosely approximated leaving a narrow space between them, as in a number of other families with monocarpellary gynoecia. This character is visible only under the microscope for some length of the ovary (Not seen at levels of Figs. 1 and 2). There is a single basal ovule as described in all taxonomic literature. Above the origin of the vascular supply for the perianth and stamens (the outer ring of eight bundles in the figures), the floral vascular cylinder bears a dorsal (marked 'D') and two ventral

(marked 'V') traces for the carpel, contracts (marked 'St') into a solid strand and runs straight upwards as an ovule trace (marked 'OV') into the basal ovule (Figs. 1 and 2).



FIGS. 1-3. Figs. 1, 2. Transverse sections of normal flower of *Elaeagnus conferta* at successively higher levels showing method of vascular supply to the basal ovule. Fig. 3. T.S. of a flower of the same species showing a marginal placentation as an unusual condition. Explanation of abbreviations is in the text. Magnification,  $\times 50$ .

In ten out of the thirty flower buds sectioned, however, there is an important difference in ovule position and in the method of vascular supply to it. The axile vascular cylinder disappears immediately above the origin of the dorsal and ventral traces for the carpel. The ovule is borne by one of the margins of the carpel close to its base, and the ventral bundle of that margin bears the ovule

trace (marked 'OV', Fig. 3) just as in typical marginal placentations. The sections are not oblique but perfectly transverse. That the marginal (or sub-marginal, if one prefers that term) position of the ovule is not due to any oblique sectioning is confirmed by the fact that merely an oblique plane of sectioning cannot by itself result in the origin of the ovule trace as a branch of the ventral bundle of the carpel.

It is very probable that the ancestors of Elaeagnaceae had marginal placentation, perhaps with more ovules than one, and with the reduction of ovule number to one, its position shifted from the lateral to a basal position. In the case of basal placentation it is convenient and economical for the plant to have the ovule trace as a direct upward continuation of the floral stele, instead of as a branch from a ventral bundle. Puri<sup>1</sup> (1952) is correct in saying that the really basal ovule in the majority of cases is a derived one from either axile or marginal placentation.

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## CONTRIBUTIONS TO THE FLORAL ANATOMY OF LINACEAE

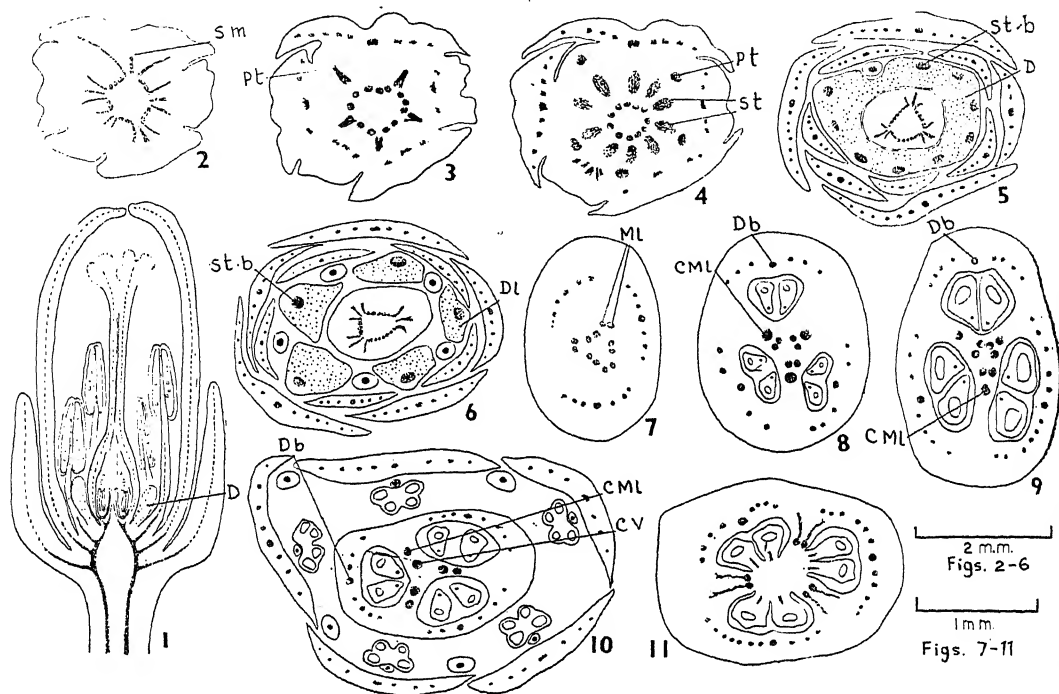
THERE are only a few published accounts on the floral anatomy of Linaceae (Narayana, 1964; Narayana and Rao, 1966, 1969, 1971). The present paper deals with the floral anatomy of *Lepidobolrys staudtii* Engl.

**Flower.**—The flower is pedicellate, pentamerous, pentacyclic, heterochlamydeous, regular, bisexual and hypogynous (Figs. 1, 10). The sepals and petals show quincuncial and imbricate aestivation respectively (Figs. 3-6, 10). The androecium consists of ten stamens of two heights, the antipetalous being shorter (Figs. 1, 10). The staminal filaments are adnate with the base of the massive disc (Figs. 1, 5). After the separation of the five antipetalous stamens, the disc becomes 5-lobed (Fig. 6), from which the antisepalous stamens separate at a higher level. The ovary is superior, 3-carpellary, syncarpous, 3-locular with two pendulous, anatropous, bitegmic ovules in each loculus (Figs. 8-11); it becomes unilocular towards the top. A placental obturator is present. The common style shows a styler canal lined by transmitting tissue. The capitate stigmas are bilobed and bear glandular hairs (Fig. 1).

**Floral anatomy.**—The pedicel shows a ring of ten to twelve vascular bundles. The sepal midrib

and lateral traces arise from a single gap (Fig. 2). The petal traces arise independently from the main stele (Fig. 3) and branch as they enter the bases of petals (Figs. 4-6, 10).

bundles (Figs. 7-10). The common ventral bundles show inverse orientation of xylem (Figs. 7-10). Each of these splits into two and forms the ovular supply (Fig. 11). The dorsal carpellary



FIGS. 1-11. *Lepidobotrys staudtii*. Fig. 1. Diagrammatic L.S. of flower showing the course of vascular bundles in the different floral parts. Figs. 2-11. Transverse sections of the flower showing the origin and distribution of the traces to the different floral parts. For explanation see text. D, Disc; Dl, Disc lobes; Sm, Sepal midrib; Pt, Petal trace; St, Staminal trace; St. b, Staminal bundle; Db, Dorsal bundle; Cml, Common median lateral bundles; ML, Median lateral bundles; Cv, Common ventral bundles.

At a higher level ten staminal traces arise in one whorl (Fig. 4). They diverge outwards and enter the ten stamens which are adnate with the intrastaminal disc at the base (Fig. 5). After the emergence of the staminal traces the main stele becomes a closed ring. As the ovary separates, three dorsal carpellary traces arise from the central stele (Figs. 5, 6). Each dorsal carpellary trace is accompanied by a pair of lateral traces which branch further and supply the ovary wall (Figs. 5, 6). The remaining vascular tissue in the centre forms six pairs of bundles (Fig. 7). Of these, the outer three pairs of bundles form the median lateral traces of adjacent carpels; these fuse at a higher level forming three common median lateral bundles (Figs. 7, 8). Towards the top of the ovary these bundles split into two and supply the ovary wall (Fig. 11). The inner three pairs of bundles also fuse to form three common ventral

bundles and some bundles in the ovary wall extend into the style and finally fade away near the base of the stigmatic lobes.

*Lepidobotrys staudtii* resembles the other investigated species of Linaceae in floral anatomical features (Narayana, 1964; Narayana and Rao, 1966, 1969, 1971). The disc is intrastaminal. The carpels are 5-traced. Placentation is anatomically parietal (Puri, 1952).

The systematic position of *Lepidobotrys* is controversial. The separation of the genera *Lepidobotrys* and *Sarcotheca* from the Linaceae and their inclusion within the Oxalidaceae was first suggested by Hallier (1921) and this was later followed by Engler and Prantl (1931). Hutchinson (1959) created a separate family Lepidobotryaceae, with *Lepidobotrys*, *Sarcotheca* and *Dapania* and placed it under Malpighiales. *Lepidobotrys* resembles the Linaceae in the following features;

quincuncial sepals, imbricate petals, ten stamens of two heights, the antipetalous being shorter, massive disc; two juxtaposed ovules per locule and the presence of an obturator. Members of Oxalidaceae differ from *Lepidobotrys* in the absence of disc, obturator and many superposed ovules in each loculus. In view of the close similarities with the members of Linaceae it would be appropriate to assign *Lepidobotrys* tentatively in the Linaceae and this concept receives further support from Metcalfe and Chalk (1950) who observed (p. 272); "*Lepidobotrys* is exceptional in many respects, but almost all its features, taken singly, can be matched in some other genus, suggesting that, while it has affinities with Linaceae, it stands somewhat apart from the other genera."

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#### EFFECT OF GA ON EXTENSION GROWTH OF PEARL MILLET (BAJRA) SEEDLINGS UNDER LOW MOISTURE LEVEL

THAT pretreatment of seeds by various chemicals involving alternate soaking and drying of seeds induces a considerable resistance to drought without interfering with germination is now well-established. Various salts, compounds and a number of growth regulating substances such as B-9, IAA, 2,

4-D are used to induce drought resistance.<sup>1-4</sup> It is surprising to note, however, that although Gibberellic acid (GA) has been extensively studied in a number of physiological processes, and also as an enzyme mobilizing hormone (EMH), its use in inducing the drought resistance has been rather nil. Therefore, the present work was undertaken to study the effect of GA as feeding media on seedling growth of 'Pearl Millet' (*Bajra*) under restricted moisture regime.

Two moisture levels were maintained, viz., 0.75 ml—restricted and 2.5 ml—optimal levels of glass distilled water (D.W.). 15 graded seeds of *Pennisetum typhoides* Stump and Conn. Cv. HB3 were germinated in sterilized petridishes containing Whatman filter-paper No. 1 circles. Simultaneously, a set was also run in which GA<sub>3</sub> solutions of 5, 10, 20 and 40 ppm were supplied as feeding media in the above two volumes, i.e., 0.75 ml GA<sub>3</sub> solutions and 2.50 ml GA<sub>3</sub> solutions as restricted and adequate feeding media levels. Germination of seeds in both D.W. and in GA media was then compared. The germination experiments were carried out at 28–30° C under normal daylight upto 96 hours and at each 24 hr interval, shoot elongation and root length of seedlings were measured. In all, 3 sets were run and the readings of 10 seedlings from all sets were taken and average calculated.

Data on extension growth of shoot of seedlings in D.W. and GA media are given in Table I. Shoot elongation is considerably arrested under low moisture regimes both in D.W. and GA media. However, seedlings incubated in GA solutions show consistently greater length than those of seedlings germinated in D.W. under both the moisture regimes. It is also noteworthy that the shoot length of GA fed seedlings at 96 hr under low moisture regime has length more or less equal to that of seedlings in D.W. under optimal moisture regime.

Root length (Table I) is also arrested under restricted moisture level. Here also, GA feeding has an enhancing effect on root length under both moisture levels. Further, as in the shoot length, root lengths of seedlings incubated in GA under restricted moisture is more or less equal to those of seedlings in D.W. under optimal moisture regime at 96 hr of germination.

Thus, it is suggested that GA can help to recover the seedlings from the effect of restricted moisture regime. This may be attributed to the action of GA on water absorptive capacity of seeds. It is known that the elongation of embryo is brought about by the imbibition of water<sup>4</sup>. The shoot and root lengths are, therefore, less in seedlings under

TABLE I

Time in hrs	D.W.	GA <sub>5</sub>	GA <sub>10</sub>	GA <sub>20</sub>	GA <sub>40</sub> ppm
<i>Shoot length (cm/seedling)</i>					
Moisture regime 0.75 ml					
24	0.11±0.01*	0.12±0.01	0.12±0.01	0.20±0.03	0.19±0.01
48	0.76±0.01	1.45±0.10	1.72±0.07	2.41±0.11	2.01±0.02
72	3.48±0.11	4.70±0.06	4.81±0.03	5.70±0.05	5.38±0.09
96	5.57±0.21	7.43±0.09	7.36±0.27	7.15±0.15	6.89±0.30
Moisture regime 2.5 ml					
24	0.62±0.01	0.71±0.06	0.72±0.06	0.75±0.03	0.83±0.02
48	3.19±0.06	3.80±0.06	3.40±0.05	4.01±0.21	4.43±0.10
72	6.14±0.08	7.20±0.16	7.13±0.10	7.29±0.02	7.66±0.11
96	7.63±0.11	8.86±0.17	8.97±0.11	8.31±0.18	9.00±0.09
<i>Root length (cm/seedling)</i>					
Moisture regime 0.75 ml					
24	0.55±0.01	0.57±0.05	0.60±0.03	0.65±0.17	0.63±0.27
48	2.85±0.20	3.13±0.05	3.45±0.01	4.51±0.01	3.94±0.01
72	6.68±0.14	8.25±0.02	8.06±0.16	8.41±0.17	9.31±0.21
96	9.13±0.60	10.94±0.24	10.23±0.20	10.20±0.35	10.91±0.40
Moisture regime 2.5 ml					
24	1.48±0.05	1.98±0.05	1.77±0.10	2.11±0.05	2.21±0.05
48	6.33±0.02	6.94±0.02	7.05±0.07	7.31±0.10	7.71±0.02
72	9.34±0.25	10.93±0.03	10.88±0.17	11.45±0.30	11.00±0.03
96	11.00±0.02	13.93±0.02	14.35±0.04	14.90±0.04	13.27±0.50

\* Standard error of the mean.

restricted moisture levels. GA, however, overcomes this water deficit by helping in the greater absorption of water as reported for chicory disc<sup>5</sup>.

The enhanced extension growth of shoot and root due to GA may also be due to increased availability of materials which supply more energy.

Further, being EMH, GA increases the hydrolytic activity of enzyme amylase which breaks down starch resulting into increased concentrations of sugars. These sugars help in maintaining the osmotic concentrations and turgidity so that more and more water can be absorbed by the seeds and seedlings. This may also cause increased shoot and root lengths.

The authors wish to thank Prof. J. J. Chinoy, Director, for his interest in this work.

Botany Department, A. B. VORA.  
University School of Sciences, K. S. DEHAL.  
Gujarat University, A. V. VYAS.  
Ahmedabad-9, September 15, 1973.

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## SHORT SCIENTIFIC NOTES

### *Rosa indica* and *Punica granatum* as New Hosts of Noctuid Moth *Achoea tirrhaea* Linnaeus in India

Semilooper moth, *Achoea tirrhaea* Linn. (Lepidoptera: Noctuidae) has been reported to suck the juice of citrus fruits (Ayyar, 1940; Pruthi and Mani, 1945). The present report records the feeding of *A. tirrhaea* larvae on two hosts, namely, *Rosa indica* (Family Rosaceae) and pomegranate, *Punica granatum* (Family Punicaceae).

During the last two years of regular survey of Horticulture Farm of Rajasthan College of Agriculture and orchards of Gulab Bagh (both at Udaipur, Rajasthan), a large number of semiloopers severely defoliating rose plants and pomegranate trees were observed. In pomegranate, these semiloopers were also found feeding on the outer bark of the twigs bearing fruits which lead to the drying of such infested twigs and subsequent premature fall of the fruits. The intensity of damage was severe during July–September but damage prolonged upto November. The pupal period was 17 to 25 days when cultured in laboratory.

The authors are thankful to the Insect Taxonomist of the Department of Entomology, Rajasthan College of Agriculture, Udaipur, for identifying the species and to Dr. B. P. Srivastava, Professor and Head of the Department, for providing necessary facilities.

Department of Entomology, V. S. BHATNAGAR,  
Rajasthan College of J. S. WAZIR,  
Agriculture,  
University of Udaipur,  
Udaipur (Rajasthan), February 11, 1974.

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### Occurrence of *Lymantria obfuscata* Walker, the Indian Gypsy Moth, as a Pest of Cacao in South India

A survey of the pests of cacao is in progress at the Central Plantation Crops Research Institute, Regional Station, Vittal. During the survey, a brownish caterpillar was observed to cause severe damage to the tender leaves of cacao. A large number of these caterpillars were collected and reared in the laboratory. The pest has been identified as *Lymantria obfuscata* Walker, Lymantridae. A preliminary note on the pest is furnished here.

The entire body of the caterpillar is brownish and is covered by brownish tufts of hairs. Hatching of eggs usually occurs during night, and, in the laboratory, the newly hatched larvae congregate on the cacao leaves but do not commence feeding until the following night. The feeding rate increases with subsequent instars and the caterpillars feed voraciously on the entire tender leaves including the veins. In the field, the first instar caterpillars usually remain on the underside of the leaves and are carried by wind from tree to tree, suspended by long threads that they spin. The later instar caterpillars feed during the night only (Rahman, 1941), and, during the day, they hide on dried leaves and twigs around the cacao tree. In severe attacks, the caterpillars defoliate all the tender leaves, retarding the growth of the tree.

The Indian gypsy moth is a pest of forest and fruit trees in certain regions of North India and is closely related to *Lymantria (Porithria) dispar* L., a destructive pest of deciduous, shade, and fruit trees in parts of Asia, Africa, Europe, and N. America (Beroza *et al.*, 1973).

This is the first record of *Lymantria obfuscata* Walker from South India.

The author is grateful to the Director, Commonwealth Institute of Entomology, London, for identifying the pest.

Central Plantation T. PREM KUMAR,  
Crops Research Institute,  
Regional Station,  
Vittal-574243, Karnataka, March 23, 1974.

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## REVIEWS AND NOTICES OF BOOKS

Annual Review of Plant Physiology (Vol. 24). (Annual Reviews, Inc., 4139, El Caminoway, Palo Alto, California 94306, U.S.A.), 1973. Pp. viii + 650. Price : U.S.A. \$ 12.00 ; elsewhere \$ 12.50.

In this volume of the *Annual Review of Plant Physiology* several aspects of topical interest are reviewed.

Two review articles, under Nutrition and Absorption, are : Electropotentials of Plant cells by N. Higginbotham and Phosphate pools, Phosphate transport and Phosphate availability by R. L. Bielecki. Under Nitrogen Metabolism, two articles, one on Protein biosynthesis by S. Zalik and B. L. Jones and another on Role of oximes in Nitrogen Metabolism in plants by S. Mahadevan, have been written.

Under Bioenergetics, three topics have been reviewed, one on : Photophosphorylation *in vivo* by W. Simonis and W. Urbach, second on Protochlorophyll and chlorophyll biosynthesis in cell-free systems from higher plants by C. A. Rebeiz and P. A. Castelfranco and the third on Photosynthetic carbonfixation in relation to net CO<sub>2</sub> uptake by C. C. Black Jr. In General Metabolism, three topics, one on Proteolytic enzymes and their inhibitions in plants by C. A. Ryan, another on Lipid metabolism in plants by P. Mazliak and the third on Metabolism of sulphate by J. A. Schiff and R. C. Hodson, have been dealt. The review article on "Plant responses to water stress" by T. C. Hsiao is of topical interest in view of the large number of studies involved in drought and related problems which are of considerable practical value.

In the topic entitled "Development", there are six reviews : Gametophyte development in ferns and bryophytes by H. Brandes ; Senescence and post-

harvest physiology by J. A. Sacter ; Dormancy in microbial spores by A. S. Sussman and H. A. Douthit ; Hormone metabolism in diseased plants by L. Sequeira ; Cytokinins as a probe of developmental processes by R. H. Hall and Gibberellins, their physiological role by R. L. Jones. It is noteworthy that the topics under development cover widely the role of bioregulators in senescence, metabolism of diseased plants and role of cytokinins and gibberellins, suggesting the importance of their role in understanding many processes associated with development.

Under Environmental Physiology, review on "Chilling injury in plants" by J. M. Lyons and "The fate of pesticides in the environment, by D. G. Crosby are of topical interest. Biochemical genetics of higher plants by O. E. Nelson and B. Burr is the special topic reviewed.

The prefatory chapter of this volume by P. J. Kramer on "Some reflections after 40 years in Plant Physiology" is one of the most thought-provoking articles. He has rightly stressed the problems involved in increase in publication and in his own words "possibly we are in more danger of being strangled intellectually by the mass of Publications than we are being strangled physiologically by air pollution". Further the need for generalists in view of the increasing trend towards specialization in a narrow field, the increasing cost involved in research and also a change in the basic approach on Physiology of whole plants have been nicely discussed which forms very interesting and illuminative reading.

K. S. K. SASTRY.

### ERRATUM

*Current Science*, Vol. 43, No. 10, Page 328 last line :  
Read, "Phenotypic Expression of Notched Kernel  
in Rice".

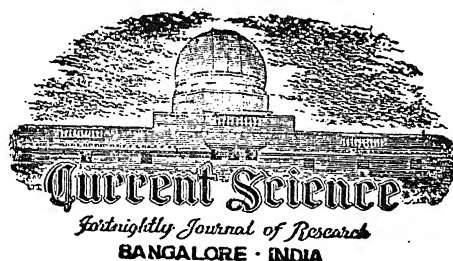
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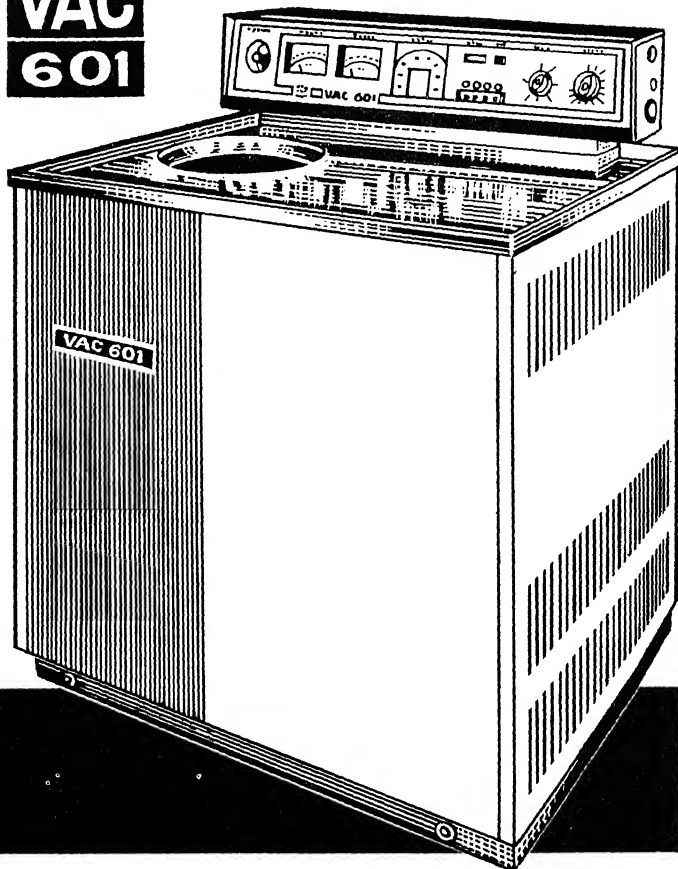


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JAISONS

# ELECTRON MICROSCOPE AND OPTICAL DIFFRACTION STUDIES ON INTERACTION OF PSYCOACTIVE DRUGS WITH BIONMEMBRANES

S. C. CHAKRAI and R. K. MISHRA

Department of Physiology, Faculty of Medical Sciences, New Delhi 110016

## ABSTRACT

Membrane structure and function were studied by optical diffraction from electron micrographs of negatively stained bionmembranes accompanied by changes in the optical diffraction pattern.

## INTRODUCTION

The interaction of psychotropic drugs with bionmembranes has been a subject of intense research. Many studies have related pharmacological effects of these drugs to their physical properties. The fluid mosaic model of membrane structure has been used to explain the activity of these drugs on bionmembranes (1,2). Electron micrographs of negatively stained isolated membranes have been used to study the structure of various emulsion and lipid vesicles (3). The effect of various drugs on membrane structure has been studied. Structural and functional changes in the bionmembrane of muscle microsome have been reported after treatment of podane antibiotic (4). The effect of podane on the structure of membrane vesicles has been reported (5). The effect of nerve excitation on peripheral membrane components by cholesterol preparation has been studied (6). Acetylcholine in lipid preparations has been studied (7). Podane studied with electron microscopy (8). By using scanning autoradiography in the electron microscope, Porter et al. (9) have reported the increase in membrane density of acetylcholine receptors in the synaptic plate.

The analysis of electron micrographs by optical diffraction (10,11) and diffractometer provides a useful method of studying the presence of periodic spacing (12). Lipid vesicles (13) and structure of cell walls (14) have been studied by diffraction of cytochrome oxidase (15), phospholipase (16) and purple membrane (17) have been studied by optical diffraction analysis of electron micrographs of negatively stained or freeze fractured preparations. By a similar analysis Chakrai (18) observed the membrane particles and alterations in size of the intercellular space sheets and junctions and junctions perpendicular. Recently we have reported evidence of structural changes in bionmembranes under the action of drugs using electron microscopy (19) and electron diffraction (20). The preliminary findings reported here concern the application of electron microscopy of negatively stained neuronal membrane fragments and the interaction of some

known neurotropic drugs on these membranes, with particular reference to the ultrastructural features and their analysis by optical diffraction.

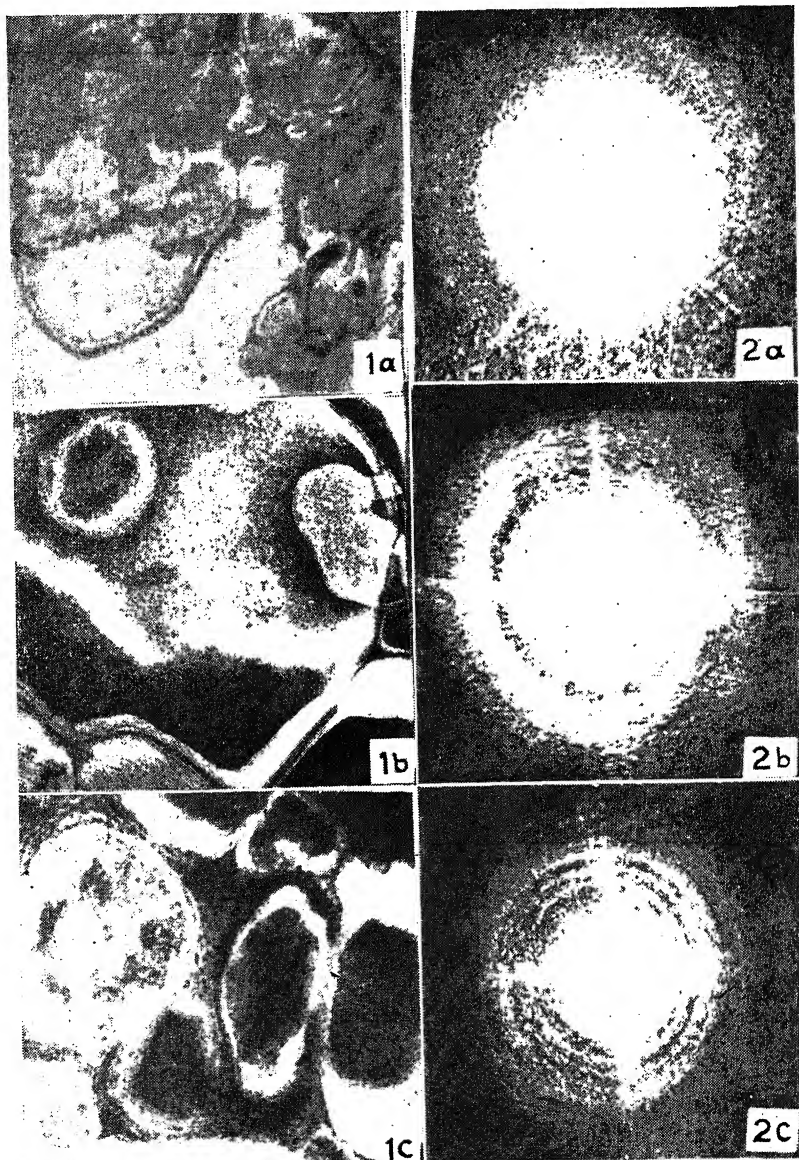
## MATERIALS AND METHOD

The neuronal membranes from rat's brain were isolated by a procedure based essentially on Neville's method and described earlier (21). The neurotropic drugs used were acetylcholine chloride, atropine sulphate, caffeine and sodium barbital, their amount used being in ratio close to their physiological dose. Micrographs were obtained with Hitachi HU 11A electron microscope using 80 KV accelerating voltage, 200  $\mu$ m condenser and 80  $\mu$ m objective apertures. Magnifications were calibrated with a mating replica (21601 mm). Membrane preparations were negatively stained with phosphotungstic acid by the droplet technique. A droplet of membrane suspension was placed on a carbon collodion covered copper grid, allowed to remain for a few seconds and then rinsed off with staining solution, and the grid blotted dry. The optical diffractometer used in these studies was of a horizontal type (22) however, one lens system was used after a design of Arundakshan (23). The light source was a Spectra Physics He Ne laser model 120, transform lens was 2 m focal length. The optical path was folded by using a reflecting mirror. The mirror did not introduce any measurable distortion. In addition to reducing the overall size of the instrument, this has the advantage that the micrograph and the ground glass viewing screen are all within easy reach of the operator. For some experiments optical microscope (Zeiss) modified after Gull (24) was also used as a diffractometer.

The electron image plates or contact copies or reduced copies made from them were used for the optical diffraction without oil immersion (25), micrograph magnifications being in the range of 80,000 to 100,000. Areas selected for diffraction were marked with opaque adhesive tape applied to non-emulsion side of the glass plate. Diffraction patterns were recorded on Kodak High Contrast film, developed in Kodak D19. The optical diffraction pattern negatives were measured on a

projectroscope. Measurement of the radial coordinate of each principal maximum was somewhat difficult, since the central peak intensity of the reflection was not clearly defined. The radial co-

selected from a somewhat larger number of patterns. The diffraction patterns were calibrated by comparison with patterns obtained from a bar grid. The diffractometer constant,



FIGS. 1-2. Fig. 1. Electron micrographs of negatively stained membrane preparations. (a) Normal neuronal membrane,  $\times 60,000$ ; (b) Membranes treated with atropine sulphate,  $\times 75,000$ ; (c) Membranes treated with acetylcholine plus atropine sulphate,  $\times 75,000$ . Fig. 2. Optical diffraction patterns obtained from electron micrographs. (a) Normal membranes; (b) Acetylcholine treated membranes; (c) Caffeine treated membranes.

ordinate of a reflection was half the distance measured across the equivalent reflections. About 10 diffraction patterns were measured. They were

$d = \text{real space distance} \times \text{reciprocal space distance}$   
 was equal to  $0.077 \text{ mm}^2$ . Photographic reproduction of diffraction patterns posed considerable

difficulty due to nonuniform and poor background. Special methods for improved reproduction of diffraction patterns and electron micrographs<sup>29-31</sup> have been described, however, these techniques have not been used in the present work.

#### RESULTS AND DISCUSSION

Some of the electron micrographs and optical diffraction patterns are shown in Figs. 1 and 2 respectively. Normal membranes appear as smooth continuous structures. On treatment with acetylcholine the membranes show areas of rarefraction and condensation of particles, which appear to be micelles, composing of membranes. Atropine sulphate which antagonises the action of acetylcholine at most of the sites produced fragmentation of membranes which was checked by combination with acetylcholine as evident from micrograph of membranes treated both with acetylcholine *plus* atropine. Appearance of membranes treated by barbitone and caffeine was considerably different from all other cases. The precise interpretation of the structures, observed in negatively stained preparations of neuronal membranes and their altered aggregation in the presence of neurotropic drugs, is problematic at present. Computer analysis and image reconstruction<sup>32</sup> would be desirable for a quantitative interpretation. Some of the qualitative inferences one may draw from these observations are: State of membrane fragment aggregation is altered by acetylcholine. The enhanced aggregation of micelles may open pores filled with water, thus facilitating diffusion across membranes and altered polarization. In general, the state of membrane organization can be modulated and can be rendered dynamically transformable by the presence of very small amount of the drugs used in these experiments.

Optical analysis of electron micrographs provides information about the nature of the order in the membrane aggregates. It shows that extensive regular arrays of the membrane subunits are not present, under the present experimental conditions of isolation and fixation, and that the order is limited to the nearest-neighbour. The commonest repeat distances observed in these studies have been summarized in Table I. Other repeat distances were approximately higher orders of these primary distances, suggesting overall random lammellar distribution of membrane fragments. Although no general conclusions can be drawn with this limited data, these studies also suggest that neurotropic drugs induce changes in the membrane association accompanied by intramolecular variation in the conformation of membrane subunits. The results obtained are in qualitative agreement with our studies on membrane-drug interaction by interference

TABLE I  
Structural spectrographic data

Specimen	Primary repeat distance nm
Normal membrane	.. 10.5±1.0
Normal membrane + Acetylcholine	.. 8.5±0.5
Normal membrane + Atropine sulphate	.. 8.2±0.5
Normal membrane + Caffeine	.. 9.5±0.4
Normal membrane + Barbitone	.. 8.1±0.5

microscopy<sup>23</sup>, electron diffraction<sup>24</sup>, circular dichroism and X-ray diffraction (being published elsewhere). Briefly, reasons for choosing various drugs used in the present study are as follows: Acetylcholine is a well-known neurotransmitter, atropine is best known cholinergic antagonist and also possesses some anticonvulsant and mild local anesthetic properties. Caffeine is known to be a powerful stimulant of the central nervous system at all levels. The barbiturates are hypnotic drugs, used for the induction of sleep, and they have a depressant effect upon the CNS.

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### THE GRAAFIAN FOLLICLE IN SOME INDIAN BATS

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THE enormous hypertrophy of the cells of the discus proligerus (cumulus oophorus) with the near complete obliteration of the antrum of the Graafian follicle has been noticed in the ovaries of some vespertilionid bats inhabiting cold and temperate regions (Wimsatt, 1944; Sluiter and Bels, 1951; Pearson *et al.*, 1952; Wimsatt and Kallen, 1957). In all these cases the Graafian follicle remains in an almost unaltered condition during the winter months when the female undergoes hibernation after coming to oestrus during autumn. Wimsatt and Kallen (1957) noticed that the hypertrophied cells of the discus proligerus of such follicles contain abundant quantities of glycogen, and considered that these modification are "an adaptation to meet the energy requirements of the ovum-follicle complex over the prolonged period of dormancy, during which time the metabolism of the animal is drastically reduced". However, the Graafian follicles of the British rhinolophid bats do not exhibit such histological peculiarities, and the cells of the discus proligerus do not hypertrophy, although these bats also undergo a long period of post-copulatory hibernation throughout winter when the Graafian follicle with a large antrum remains almost unchanged in the ovary (Matthews, 1937).

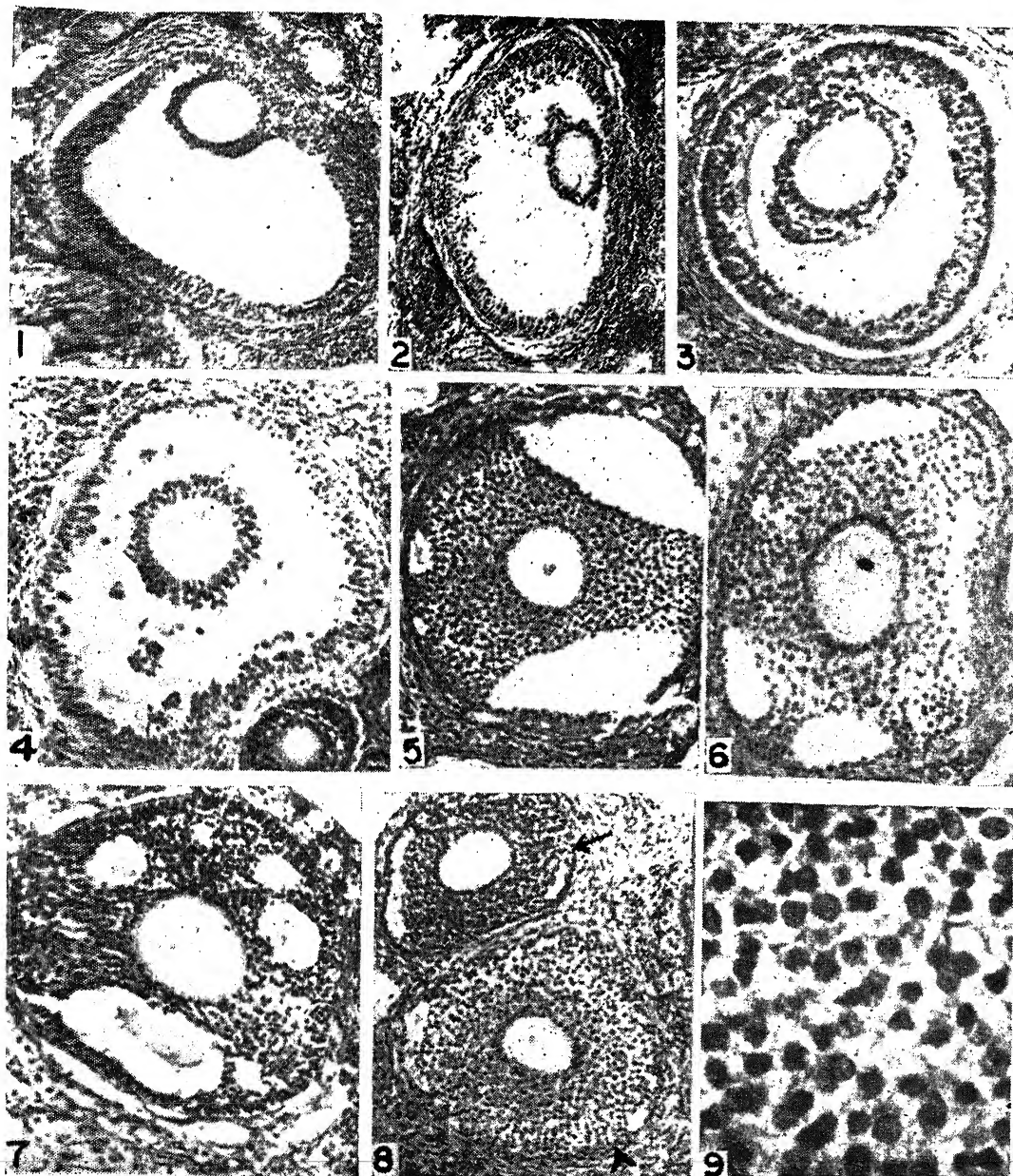
Since the structure of the Graafian follicle varies so much even amongst the hibernating bats inhabiting cold climates, it was felt that it would be interesting to make a comparative study of the Graafian follicles of some tropical bats belonging to different families. The present report embodies the description of the Graafian follicle of *Pteropus*

*giganteus giganteus* (Pteropidae), *Megaderma lyra lyra* (Megadermatidae), *Rhinolophus rouxi* (Rhinolophidae), *Hipposideros speoris* (Hipposideridae), *Pipistrellus ceylonicus chrysothrix*, *P. minimus minimus* and *P. dormeri* (all belonging to Vespertilionidae). None of these species undergoes hibernation as noticed in the bats of cold and temperate regions.

Figures 1-7 illustrate the structure of the fully developed Graafian follicle in the seven species of bats studied here. Whereas the Graafian follicle of *Pteropus*, *Megaderma*, *Rhinolophus* and *Hipposideros* presents a picture typical of the Graafian follicle of most mammals in possessing a large antral cavity and in having the ovum surrounded by one or two layers of small cumulus cells (Figs. 1-4), the mature follicles of the vespertilionid species (Figs. 5-7) studied here have a different structure, and are nearly similar to those of some of the hibernating vespertilionids inhabiting cold climates. In all the vespertilionids studied here the cells of the discus proligerus undergo enormous hypertrophy, and cellular bridges extend from the enlarged discus to the granulosa layers resulting in the reduction of the antral cavity to one or a few small spaces. Further, the hypertrophied cells of the discus proligerus contain numerous fluid-filled vacuoles (Fig. 9). The peculiar appearance of the mature follicle of the vespertilionid bats is, at least, partly due to the accumulation of secretions within the cumulus cells themselves. This is evident from the fact that the follicle cells are small and compactly arranged at the multilaminar and early vesicular stages of development of the follicle. Hence,

these follicles appear as dark bodies in stained sections (Fig. 8). The enormous increase in the size of the follicle after this stage is due mostly to the enlargement of individual follicle cells accom-

panied by an accumulation of secretions inside the cells without an appreciable increase in the number of cells. Hence, the mature follicles are lightly stained and have vacuolated cells (Fig. 8). In the



FIGS. 1-9. Figs. 1-7. Photomicrographs of the Graafian follicles of *Pteropus giganteus giganteus* ( $\times 140$ ), *Megaderma lyra lyra* ( $\times 160$ ), *Rhinolophus rouxi* ( $\times 220$ ), *Hipposideros speoris* ( $\times 140$ ), *Pipistrellus ceylonicus chrysothrix* ( $\times 160$ ), *Pipistrellus mimus mimus* ( $\times 200$ ) and *Pipistrellus dormeri* ( $\times 200$ ) respectively. Fig. 8. Section of the ovary of *Pipistrellus mimus mimus* showing a multi-laminar follicle with compactly arranged follicle cells (arrow) and a preovulatory follicle with hyper-trophied cumulus cells (arrow head) ( $\times 106$ ). Fig. 9. A part of the discus proligerus of *Pipistrellus ceylonicus chrysothrix* to show the enlarged vacuolated cells ( $\times 860$ ).



other bats the secretions produced by the follicle cells escape out of the cells and accumulate in the antral cavity so that the great increase in the size of the follicle is due to the enlargement of the antrum. Apparently, the unique histological changes resulting in the hypertrophy of the cumulus cells is a feature characteristic of some of the vespertilionids amongst the bats irrespective of their geographical location and irrespective of whether they undergo a protracted post-copulatory hibernation or not. The Graafian follicles of bats belonging to no other family have been so far shown to

exhibit these modifications of the cells of the discus proligerus.

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### MINERALOGY AND CHEMISTRY OF ASBESTOS FROM HOLENARASIPUR SCHIST BELT : MANGALAPUR AREA

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#### ABSTRACT

Asbestos from the Mangalapur area of Holenarasipur Schist belt has been identified as anthophyllite contrary to the earlier report as tremolite. The serpentines in this area are 6-layer orthoantigorites and the chlorites are 14 Å, 11b polytypes of variable Si/Al ratios. The anthophyllite is derived from the alteration of antigorite, chlorite and talc by the hydrothermal metamorphism of the ultrabasic intrusives.

**A**SBESTOS, a mineral of wide variety of uses, is found widely scattered in different parts of the Precambrian of Mysore. The difficulty in identifying the types of asbestos has led to erroneous conclusions regarding its geology and genesis. Since the physicochemical methods are the only means by which the asbestos minerals can be identified conclusively, the present series of investigations have been taken up. Amphibole asbestos forms the major exploitable variety in the State. One of the major workable deposits of asbestos is confined to Holenarasipur schist belt, south of 13° latitude and west of 76° 20' E longitude. The asbestos from this area have been differently identified as tremolite-actinolite, anthophyllite and chrysotile. A systematic study of asbestos and the coexisting minerals from the southern limb of the Y-shaped schist belt, south of the river Hemavathi is undertaken. The present report is confined to Mangalapur area, north of the old Holenarasipur-Channarayapatna road and south of the river Hemavathi.

The geology of Holenarasipur schist belt has been repeatedly studied by many in the past<sup>1-5</sup>. The principal rock types are: the metamorphosed ultramafites, kyanite-staurolite schists and hornblende schists. The occurrence of asbestos is confined to the altered ultramafites. The veins in dis-

connected lenses with a general N-S trend occur in them within a span of 30 miles.

In Mangalapur region asbestos are of varying habits, fine as well as massive fibres of differing length (a few inches to 6 feet), varying in colour (grey, brown and white) and also in strength. The massive woody type and its host rock are associated with the asbestos. The minerals coexisting with asbestos are: carbonates such as dolomite, calcite and magnesite; chlorites of flaky, coarse and massive habits; serpentines with magnetite grains. The chlorites are contiguous with the serpentinites and is abundant towards the kyanite-staurolite schists. The asbestos bearing horizons are confined to the vicinity of serpentinite bodies in the field. The lithological features of the schist belt suggest that subsequent to the ultramafic intrusion into the pelitic sediments, the terrain has been subjected to dynamic metamorphism and deformation.

#### MINERALOGY OF THE ASBESTOS

The chemical analysis of a number of asbestos samples from Mangalapur area showed that they are all anthophyllites as against the reported occurrence of tremolite asbestos<sup>3,4</sup>. Mountain wood and its host rock are also found to be anthophyllites. The typical analyses of asbestos, mountain wood and its host rock are given in

Table I. The X-ray data show that all the three are orthorhombic confirming the above conclusion.

TABLE I  
Chemical composition of asbestos

	Fibrous asbestos	Mountain wood	Massive Anthophyllite
SiO <sub>2</sub>	55.09	58.15	57.20
TiO <sub>2</sub>	0.00	0.00	0.00
Al <sub>2</sub> O <sub>3</sub>	2.07	2.27	1.32
Fe <sub>2</sub> O <sub>3</sub>	3.19	6.52	0.61
FeO	13.68	6.41	17.03
MnO	0.19	0.21	0.11
MgO	21.62	21.55	20.79
CaO	0.93	2.22	0.45
Na <sub>2</sub> O	0.15	0.20	0.12
K <sub>2</sub> O	0.21	0.00	0.01
H <sub>2</sub> O <sup>+</sup>	2.43	2.85	2.35
H <sub>2</sub> O <sup>-</sup>	0.34	0.16	0.16
CO <sub>2</sub>	0.21	0.00	0.00
TOTAL	100.11	100.54	100.15
a (Å)	18.53	18.48	18.54
b (Å)	17.98	18.09	17.98
c (Å)	5.31	5.30	5.33
β	90°	90°	90°

Number of ions on the basis of 24 (0) atoms

Si	7.785	7.863	7.996
Al	0.215	0.137	0.004
Al	0.130	0.224	0.213
Ti	0.000	0.000	0.000
Fe <sup>3+</sup>	0.343	0.661	0.064
Mg	4.556	4.330	4.332
Fe <sup>2+</sup>	1.618	0.723	1.991
Mn	0.023	0.024	0.013
Na	0.028	0.052	0.033
K	0.058	0.000	0.002
Ca	0.141	0.321	0.067
(OH)	2.296	2.565	2.193

The serpentinite boulders occurring close to one of the asbestos pits have been examined. The minerals of the serpentinite have been identified by the optical methods (Fig. 1). The minerals

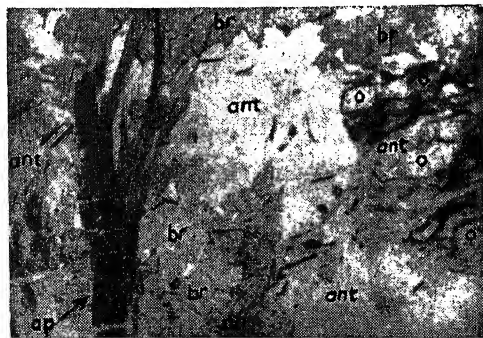


FIG. 1. Serpentinite. ant—antigorite, an—anthophyllite, br—breunnerite, O—Olivine with streaks of magnetite surrounding.

are antigorite, anthophyllite, magnesite, magnetite and relict olivine. The minerals have been separated using magnetic and gravity methods. The analytical data are given in Table II. In the antigorite, there is considerable substitution of Mg by Fe<sup>2+</sup>. The X-ray powder diffraction data given in Table IV show that this is a 6-layer orthoserpentine. All the observed reflections could be indexed as per data furnished by Gillerly<sup>6</sup>. In the magnesite also there is substitution of Mg by Fe<sup>2+</sup> suggesting that it is breunnerite. The substitution is true even in the dolomite (4% by wt. of FeO) occurring separately associated with anthophyllites. The coexistence of anthophyllite with the antigorite and magnesite is very characteristic in this area.

TABLE II

Chemical composition of serpentinite minerals

	Anti- gorite	Breun- nerite	Antho- phyllite
SiO <sub>2</sub>	41.65	..	54.58
TiO <sub>2</sub>	0.00	..	0.00
Al <sub>2</sub> O <sub>3</sub>	3.80	..	2.53
Fe <sub>2</sub> O <sub>3</sub>	0.61	0.00	0.47
FeO	8.32	9.73	14.76
MnO	0.08	0.09	0.03
MgO	33.88	40.15	24.69
CaO	0.17	0.26	0.56
Na <sub>2</sub> O	0.02	0.00	0.18
K <sub>2</sub> O	0.01	0.00	0.07
H <sub>2</sub> O <sup>+</sup>	10.98	0.08	1.97
H <sub>2</sub> O <sup>-</sup>	0.32	..	0.08
CO <sub>2</sub>	0.39	49.82	0.00
TOTAL	100.18	100.13	99.92

No. of ions	Basis 9(0) atoms	6 (0) atoms	24 (0) atoms
Si	2.025	..	7.672
Al	..	..	0.328
Al	0.218	..	0.005
Ti	0.000	..	0.000
Fe <sup>3+</sup>	0.022	0.000	0.005
Fe <sup>2+</sup>	0.339	0.239	1.735
Mn	0.003	0.002	0.004
Mg	2.459	1.759	5.180
Ca	0.009	0.008	0.085
Na	0.002	0.000	0.005
K	0.001	0.000	0.001
(OH)	1.785	..	1.850
CO <sub>2</sub>	..	1.997	..

The cell dimensions for the anthophyllite are:  $a=18.52$  Å;  $b=17.98$  Å;  $c=5.33$  Å;  $\beta=90^\circ$ .

The chlorites are of variable chemical composition of differing Si/Al ratios. Analyses show that



TABLE III  
Chemical composition of chlorites

	Chlorite (Ripidolite)	Talc-chlorite
SiO <sub>2</sub>	26.71	39.20
TiO <sub>2</sub>	0.00	0.00
Al <sub>2</sub> O <sub>3</sub>	21.85	9.31
Fe <sub>2</sub> O <sub>3</sub>	0.08	0.00
FeO	14.76	5.75
MnO	0.03	0.04
MgO	24.30	34.27
CaO	0.00	0.28
Na <sub>2</sub> O	0.08	0.02
K <sub>2</sub> O	0.01	0.00
H <sub>2</sub> O <sup>+</sup>	12.09	11.19
H <sub>2</sub> O <sup>-</sup>	0.12	0.00
CO <sub>2</sub>	0.00	00.00
TOTAL	100.03	100.06

Number of ions on the basis of 36 (O) atoms

Si	5.308	} 8.000	7.501	} 8.000
Al	2.692		0.499	
Al	2.422	} 12.110	1.598	} 12.355
Ti	0.000		0.000	
Fe <sub>3</sub> <sup>+</sup>	0.012		0.000	
Fe <sub>2</sub> <sup>+</sup>	2.451		0.920	
Mn	0.005		0.006	
Mg	7.188		9.763	
Ca	0.000		0.058	
Na	0.031		0.005	
K	0.001		0.000	
(OH)	16.03		14.31	

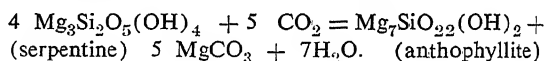
the composition vary from ripidolite-heridanite to talc-chlorite. The data for the two extreme members are presented in Table III. The X-ray data (Table IV) of clinocllore composition show that it is 14Å, II b polytype<sup>7,10</sup> suggesting that they are not low temperature alteration products. The talc-chlorite (Table III) occurs as nodules within the asbestos veins. Their mode of occurrence strongly suggest that they are the relicts of a reaction that gave rise to anthophyllite asbestos. It may also be mentioned that occasionally talc occurs with dolomite-anthophyllite pair.

#### GEOCHEMISTRY AND GENESIS

The genesis of asbestos is closely connected with the hydrothermal metamorphism of the ultramafites in the Holenarasipur schist belt. In the normal course, metamorphism of ultramafites gives rise

to serpentines of which chrysotile is one of the polymorphs. In the present instance, instead of chrysotile the anthophyllite asbestos happens to be the final reaction product. This suggests that a separate sequence of reaction must have taken place in this area.

Based on the available experimental data on the stability relations of minerals and hydrothermal synthesis of anthophyllite<sup>8,9</sup>, the absence of orthopyroxene (particularly enstatite) and cordierites from the host rocks of asbestos and based on the coexisting minerals with the anthophyllites, we postulate the possible sequence of reactions. After the intrusion of ultramafites into the pelitic sediments, the central portion of the intrusive transformed into serpentinite with added water. The peripheral portions, on the other hand, reacted with the alumina rich sediments leading to the formation of chlorites in presence of water. Chlorites of such an origin can have varying Si/Al ratio. The serpentines and the chlorites thus formed underwent a second set of reactions under hydrothermal conditions. The total pressure for such a reaction is built up both by  $P_{H_2O}$  and  $P_{CO_2}$ , with  $P_{H_2O}$  being smaller which is more conducive for the formation of anthophyllite<sup>8</sup>. The serpentine underwent the following reaction in presence of CO<sub>2</sub>:



giving rise to anthophyllite and magnesite. Because of the iron-rich conditions in the area, substitution of Mg by Fe<sup>2+</sup> took place.

The other set of reaction leading to the formation of anthophyllite and talc is by the breakdown of alumina poor chlorites which are known to be unstable under the hydrothermal conditions. The nuclei for the anthophyllite crystallisation are provided by the reaction given above. It is also known that talc can give rise to anthophyllite<sup>8,9</sup>.

The experimental data show that anthophyllites are formed under restricted P-T conditions (P = 500 to 2000 bars; T = 550 to 760°C) with narrow stability range<sup>8</sup>. The 14 Å chlorites, particularly the II b polytype are stable under the above P-T conditions. This suggests that anthophyllites of variable habits occurring as disconnected lenses replacing the ultramafites, have been derived from the alteration of antigorite, chlorite and talc formed during hydrothermal metamorphism. The deformational features of the rock types also suggest the role of shearing stress in the formation of anthophyllite asbestos.

TABLE IV

X-ray powder diffraction data of antigorite and clinochlore

Antigorite			Clinochlore		
d (Å)	Intensity	hkl	d (Å)	Intensity	hkl
7.08	>100	006	14.09	75	001
4.61	3	020	7.06	100	002
3.86	3	026	4.718	65	003
3.554	>100	0,0,12	3.536	100	004
3.300	5	029	2.825	40	005
3.124	8	0,2,10	2.584	45	20 $\bar{2}$
3.047	10	0,0,14	2.537	80	201
2.647	10	200	2.438	65	20 $\bar{3}$
2.523	70	205	2.376	40	202
2.439	5	207	2.252	35	20 $\bar{4}$
2.399	10	208	2.057	20	20 $\bar{5}$
2.256	3	2,0,10	1.998	50	204
2.207	3	2,0,12	1.876	25	20 $\bar{6}$
2.016	50	2,0,14	1.819	30	205
1.878	5	2,0,16	1.657	15	206
1.775	10	0,0,25	1.562	40	20 $\bar{8}$
1.726	15	310	1.540	50	060
1.704	20	2,0,20	1.502	25	062
1.570	10	2,0,22	1.414	15	0,0,10
1.541	20	060	1.392	35	208
1.525	8	0,0,28	1.208	10	0,4,10
1.502	10	066	1.180	25	0,0,12
1.472	8	068			
1.421	5	0,0,30			
1.322	20	400			
1.290	3	0,6,18			
1.254	5	0,0,34			

$a = 5.29 \text{ \AA}$ ;  $b = 9.25 \text{ \AA}$ ;  $c = 42.66 \text{ \AA}$ ;  $\beta = 90^\circ$   
 $D = 2.634$ .

$a = 5.32 \text{ \AA}$ ;  $b = 9.24 \text{ \AA}$ ;  $c = 14.27 \text{ \AA}$ ;  $\beta = 97^\circ$ ;  
 $\text{csin } \beta = 14.06 \text{ \AA}$ ;  $D = 2.804$ .

The clinochlore composition corresponds to—  
 $(\text{Al}_{1.85} \text{Fe}^{3+}_{1.18} \text{Fe}^{2+}_{2.31} \text{Mg}_{7.88}) (\text{Si}_{5.73} \text{Al}_{2.27}) (\text{OH})_{16.1}$

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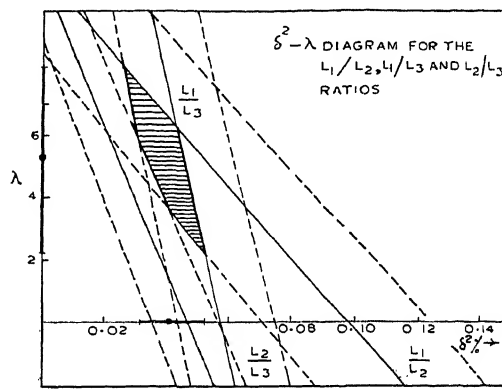
## LETTERS TO THE EDITOR

ACCURATE DETERMINATION OF THE M1-E2  
MIXING RATIO FOR THE 39.58 keV  
TRANSITION IN  $^{129}\text{Xe}$  EMPLOYING NUCLEAR  
STRUCTURE PARAMETER

THE experimental L-sub-shell ratio is a powerful tool for the accurate determination of multiple admixtures. Geiger *et al.*<sup>1</sup> have measured the L-conversion intensity ratios of the 39.58 keV transition in  $^{129}\text{Xe}$  using 1 m radius iron free double-focussing spectrometer. With these data, we have carried out the analysis for the penetration parameter and mixing ratio (Fig. 1). The details of the present analysis are presented in our previous paper<sup>2</sup>. The theoretical tabulations of Hager and Seltzer<sup>3</sup> for the conversion coefficients and penetration functions<sup>4</sup> have been employed. The best values for  $\lambda$  and  $\delta^2$  have been obtained from the figure by the centre of gravity of the shaded area. The figure also incorporates the error bands.

From the figure we find a region of overlap for the mixing ratio  $\delta^2$ ,  $0.041^{+0.013}_{-0.009}$  and an experimental value for the penetration parameter  $\lambda$  as  $5.3^{+2.8}_{-3.1}$ . The mixing ratio determined from the

tions of Sliv and Band. A good overlap with the theoretical values can be obtained if we take into account the penetration effects also by considering the value of the present penetration parameter.



The results of the present analysis using the Hager and Seltzer tables and that of Geiger *et al.* who have used the tables of Sliv and Band are given hereunder.

Nucleus	Energy	Spin Seq.	E2 Admixture (%) $\delta^2$	Penetration parameter $\lambda$ (Present work)
$^{129}\text{Xe}$	39.58 keV	d3/2 — s1/2	$0.075 \pm 0.025$ (Geiger <i>et al.</i> )	
			$0.041^{+0.013}_{-0.009}$ (Present work)	$5.3^{+2.8}_{-3.1}$

region of overlap differs considerably from the  $\delta^2$  value of Geiger *et al.* who have employed the theoretical values for the sub-shell ratios due to Sliv and Band<sup>4</sup>. The fact that Geiger *et al.* have found inconsistency between their experimental ratios and the predictions of Sliv and Band indicates that the anomaly in the internal conversion coefficients of this transition are due to the nuclear structure effects in the internal conversion process of the 39.58 keV transition in  $^{129}\text{Xe}$ , which has been confirmed from the large value of the penetration parameter obtained in the present analysis. The present analysis gives a very reliable value for the mixing ratio  $\delta^2$  with very small associated errors and a reasonably good range for the penetration parameter  $\lambda$ . The poor overlap of the mixing ratio in the earlier report is mainly due to the inaccuracies involved in the theoretical tabula-

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### SOME NEW FLUOROBERYLLATES

FLUOROBERYLLATES have hitherto been prepared by dissolving oxide or carbonate of beryllium in hydrofluoric acid with the requisite quantities of hydroxide, oxide or carbonate of other metals. Slightly acidic medium is more favourable for the formation of these fluoroberyllates. Isolation of free fluoroberyllic acid<sup>1</sup> has facilitated the preparation of these fluoroberyllates in that simply treatment of the acid with the oxide or carbonate of a metal would produce the fluoroberyllate. Care should, however, be taken to avoid complete neutralisation of the acid. Recently several new fluoroberyllates have been reported<sup>2</sup>. This paper deals with the preparation of a few rare earth fluoroberyllates, alkyl fluoroberyllates and fluoroberyllates of organic amines.

Fluoroberyllic acid used was prepared by the ion exchange method<sup>1</sup>. Rare earth carbonates were prepared by passing carbon dioxide through a suspension of the oxides in water.

Organic amines were distilled before use. The following organic amines were used: (1) Aniline; (2) Pyridine; (3) Quinoline and (4) Triethyl amine. Methyl and Ethyl iodides used were of G.R. grade.

Fluoroberyllic acid (6 N) was taken in a polythene beaker and the rare earth carbonates were

over conc.  $H_2SO_4$ . After a few days crystals separated. These were washed in alcohol and dried. In this way ceric fluoroberyllate (yellow), lanthanum fluoroberyllate (light yellow) were obtained. Europium beryllate was obtained as a white precipitate during neutralisation.

Methyl and Ethyl fluoroberyllates were prepared from the respective iodides and silver fluoroberyllates. Alcoholic solutions of the alkyl halide were taken and shaken with silver fluoroberyllate for 15 minutes then filtered in suction. The alcoholic solution was placed on a water bath till the alcohol is removed. The methyl and ethyl fluoroberyllates being high boiling were left over. They are colourless liquids slightly soluble in water.

Fluoroberyllic acid (6 N) and organic amines were mixed in 1 : 2 molar proportion. The mixed solutions were evaporated in a vacuum desiccator over conc.  $H_2SO_4$ . The crystals were redissolved in water. On adding alcohol to this aqueous solution crystals of organic amine fluoroberyllates separated. Aniline, pyridine, quinoline and triethyl amine fluoroberyllates were prepared in this way. They are colourless crystals soluble in water but not in alcohol and ether.

Cerium and lanthanum were estimated by precipitating as oxalate and igniting to oxide. Europium was estimated as sulphate.

TABLE I  
Results of analysis

Compounds	F%		Be%		N%		R.E. %	
	obs.	cal.	obs.	cal.	obs.	cal.	obs.	cal.
Cerium fluoroberyllate ..	33.56	33.64	4.02	3.98	..	..	41.22	41.32
Lanthanum fluoroberyllate ..	32.72	32.80	3.97	3.88	..	..	39.86	39.99
Europium fluoroberyllate ..	32.39	32.43	3.91	3.84	..	..	43.18	43.24
Aniline fluoroberyllate ..	27.79	27.87	3.23	3.29	10.20	10.25	..	..
Pyridine fluoroberyllate ..	31.11	31.02	3.62	3.67	11.39	11.42	..	..
Quinoline fluoroberyllate ..	21.89	22.02	2.64	2.60	7.98	8.11	..	..
Triethyl amine fluoro- beryllate ..	26.23	26.29	3.16	3.11	9.60	9.68	..	..
Methyl fluoroberyllate ..	66.13	66.06	7.76	7.82	..	..	..	..
Ethyl fluoroberyllate ..	53.09	53.14	6.33	6.29	..	..	..	..

added a little at a time till effervescence ceased. Complete neutralisation was not found desirable. The solution was left a bit acidic. It was filtered and allowed to crystallise in a vacuum desiccator

Beryllium was estimated by precipitating as  $Be(OH)_2$  and igniting to  $BeO$ . For estimating fluorine, beryllium was precipitated as  $Be(OH)_2$  with ammonia and removed by filtration. In the

filtrate, fluorine was estimated by precipitation as  $\text{CaF}_2$  in ammoniacal medium and weighing as  $\text{CaF}_2$  after ignition. Nitrogen was estimated by Kjeldahl's method or by Dumas method.

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#### GRAVIMETRIC ESTIMATION OF SILVER AS DIAMMINESILVER (I) TETRAISOTHIOCYANATODIANILINECHROMATE (III)

It has been reported that silver (I) cannot be estimated by tetraisoithiocyanatodanilinechromate (III) anion in presence of  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$  and  $\text{SCN}^-$  ions<sup>1</sup>. In this paper, we report that it can be estimated in presence of  $\text{Cl}^-$  and small amount of  $\text{Br}^-$  ions as diamminesilver (I)-tetraisoithiocyanatodanilinechromate (III).

compound rendered it possible to estimate silver (I) in minute quantities. The ratio of silver and the precipitated compound is 1 : 5.67 (Grav. Factor = 0.1764). A number of experiments involving solutions containing varying amounts of silver and several metal ions confirmed the reliability of the method.

#### Experimental

**Reagent solution.**—(Aniline) $\text{H}[\text{Cr}(\text{NCS})_4(\text{aniline})_2]$  was prepared by the method of Ganguly<sup>2</sup>. Freshly prepared saturated solution of the reagent in alcohol was used for all precipitations.

**Procedure.**—Precipitate out Ag as  $\text{AgCl}$  in the silver solution by usual method. Collect the precipitate on a sintered crucible or Whatman filter paper. Wash with 0.1 N  $\text{HNO}_3$ . Dissolve the precipitate in liquor ammonia (1 : 1). Add freshly prepared saturated solution of the reagent in alcohol to the silver solution in slight excess to precipitate out pink rose coloured precipitate,  $[\text{Ag}(\text{NH}_3)_2][\text{Cr}(\text{NCS})_4(\text{aniline})_2]$ . Thereafter, keep in an ice-bath for 30 min with stirring, filter off on a weighed filtering crucible (Gooch or sintered), wash with cold water, alcohol and finally with ether and dry at  $110^\circ\text{C}$ .

TABLE I  
Separation of silver from foreign ions

Expt. No.	Ag taken mg	foreign	ions added mg	Wt. of ppt. mg	Ag found	Error mg
1	18.24	Nil	..	103.0	18.18	0.06
2	15.63	Nil	..	88.4	15.60	0.03
3	18.24	$\text{Cu}^{2+}$	15.34	103.1	18.20	0.04
4	18.24	$\text{Mn}^{2+}$	14.41	103.0	18.18	0.06
5	18.24	$\text{Ni}^{2+}$	19.42	103.3	18.23	-0.01
6	15.63	$\text{Co}^{2+}$	14.62	88.4	15.60	-0.03
7	15.63	$\text{Pd}^{2+}$	23.99	88.6	15.63	-0.00
8	15.63	$\text{Au}^{3+}$	9.21	88.5	15.62	-0.01
9	15.63	$\text{Br}^-$	8.54	88.4	15.60	-0.03
10	15.63	$\text{Br}^-$	31.84	86.1	15.19	-0.44

Freshly prepared alcoholic solution of (aniline)  $\text{H}[\text{Cr}(\text{NCS})_4(\text{aniline})_2]$  with  $\text{Ag}(\text{NH}_3)_2\text{Cl}$  solution gives pink rose precipitate,  $[\text{Ag}(\text{NH}_3)_2][\text{Cr}(\text{NCS})_4(\text{aniline})_2]$  which is insoluble in water and common organic solvents and easy to wash. The high molecular weight (m.w. = 612) of the precipitated

Amounts of 15–50 mg of Ag (corresponding to a weight of precipitate of 85–284 mg) have been successfully determined with errors of not more than 1%. Many cations do not interfere, e.g.,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pd}^{2+}$ ,  $\text{Au}^{3+}$ . Small amounts of  $\text{Br}^-$  also do not interfere. Cations having their

chlorides soluble in water will not interfere. Anions which form compounds more insoluble than AgCl (AgBr) will interfere, e.g.,  $I^-$  and  $SCN^-$ .

In the analysis of  $[Ag(NH_3)_2][Cr(NCS)_4(\text{aniline})_2]$ , silver was estimated as AgCl gravimetrically, chromium volumetrically and nitrogen by Kjeldahl method. The following values have been found :

Ag 17.51 (17.64); Cr 8.38 (8.50) and N (18.31) (18.30); calculated values in parentheses.

This method appears to be especially suited for semimicro and micro estimations of chlorine in water and various organic compounds.  $AgNO_3$  is added to the solution containing  $Cl^-$  ions to precipitate AgCl which may be dissolved in liquor ammonia (1:1) and the reagent may be added dropwise to silver complex solution to precipitate out  $[Ag(NH_3)_2][Cr(NCS)_4(\text{aniline})_2]$ . Chlorine in organic compounds can be converted into a chloride by the usual procedure and estimated in the manner described earlier.

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#### SPECTROPHOTOMETRIC AND ANALYTICAL STUDIES OF THE U(VI) COMPLEX WITH 4, 6-DIHYDROXY-3', 4'-DIMETHOXYAURONE

RECENTLY Dhar and Jain<sup>1</sup> observed that 4, 6-dihydroxy-3', 4'-dimethoxy aurone produces an orange red spot with U(VI) on a paper chromatogram. The nature and the composition of this coloured complex has now been investigated.

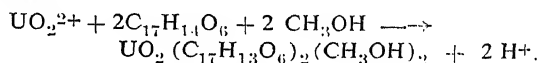
**Experimental.**—Uranyl nitrate (AnalaR) was used for the preparation of standard solution of uranium which was estimated by the usual gravimetric procedure<sup>2</sup>. The ligand 4, 6-dihydroxy-3', 4'-dimethoxyaurone was prepared according to the method given by Jain *et al.*<sup>3</sup> and King *et al.*<sup>4</sup> and was purified by repeated crystallizations until it gave a single spot in TLC solvent system: ethyl formate-formic acid-toluene (20:6:25) and a constant melting point of 220° (Lit<sup>3</sup>, 220–21°). A standard solution of the reagent was prepared in 95% ethanol. Optical density measurements were carried out with the Beckman DB Spectrophotometer and pH measurements with Philips 9405 L pH meter. Job's method of continuous variations as modified by Vosburgh and Cooper<sup>5</sup> and the slope ratio method

of Harvey and Mannings<sup>6</sup> were employed to determine the molar ratio. Since the spectrum of the ligand and the complex showed separately maxima at 410 nm and at 410 and 470 nm respectively, the optical density measurements were recorded at 490 and 520 nm. In continuous variations method (using  $0.5 \times 10^{-3}$  M solutions) the optical density measurements were also carried out at 490 and 520 nm (pH 4.0). Ligand solutions of appropriate strength were taken as reference in order to correct the observed optical density for no reaction of the constituents. Similarly  $0.66 \times 10^{-3}$  M solutions were used and optical density measurements carried out at 520 nm and pH 4.0; 95% ethanol was taken as reference. All the three curves showed a stoichiometry of 1:2 (Metal:Ligand).

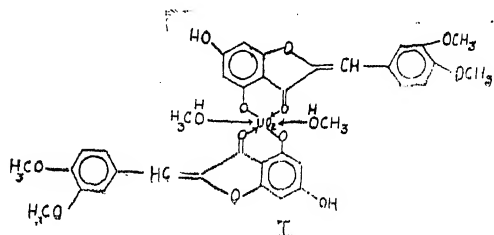
The above result is supported by slope ratio method also. Two series of solutions were prepared using  $0.5 \times 10^{-3}$  solutions of the aurone derivative and U(VI) salt solution. In one series U(VI) concentration was varied keeping ligand concentration constant but in sufficient excess and in the second series ligand concentration was varied keeping metal ion concentration constant but in sufficient excess. The optical densities of solutions were recorded at 490 nm using alcohol (95%) as reference.

The metal complex was prepared in the solid state by mixing the methanolic solutions of uranyl nitrate and the aurone derivative in the molar ratio of 1:2. The resulting solution on concentration yielded a deep red coloured solid which was purified by repeated crystallization and dried over anhydrous calcium chloride in vacuum. Uranium was estimated in this complex as  $U_3O_8$  by ignition at about 1000° C (Found :  $UO_2$ , 28.3%, C, 44.7%, H, 4.4%;  $UO_2C_{36}H_{34}O_{14}$  requires  $UO_2$ , 28.1%, C, 45.0%, H, 3.5%); IR:  $\nu_{max}$  1580 (C=O), 3370  $cm^{-1}$  (O-H) both of which are lower values than those of the ligand.

**Results and Discussion.**—The results suggest that the complex formation may be represented as follows:



The structure (I) may be proposed for the complex.



I.R. NMR and magnetic measurements studies are being conducted to establish the bonding and structure in detail.

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#### FATTY ACID COMPOSITION OF KUSUM (*SCHLICHERA TRIJUGA*) SEED OIL BY GAS-LIQUID CHROMATOGRAPHY

THE fatty acid composition of Kusum seed oil was done long before the introduction of GLC. The present communication reports on the fatty acid composition of Kusum seed oil by GLC.

The dried seeds were ground and extracted with chloroform methanol (1:2). The bulk of the solvent was removed. The oil was in yield of about 34.0%. The iodine value (Wijs' method) of the oil was found to be 62.15. Saponification of oil, extraction of fatty acids and formation of methyl esters were done according to T. P. Hilditch<sup>2</sup>. The analyses were carried out by F and M Model 700 R Dual-column gas chromatograph with a flame ionisation detector using a 10% DEGS column on Gas-chrom Z (Applied Science Laboratories, Inc., U.S.A) at 180°C and 3% SE 30 column on the same support at 210°C.

Chromatographic peaks of methyl esters were identified by plotting the log of retention times against the number of carbon atoms in the chain<sup>3</sup>. The peak areas were calculated by the method of triangulation<sup>4</sup>. The data calculated as wt. per cent of fatty acids is given in Table I.

From the composition it is observed that palmitic, oleic and linoleic acids are the major components of the mixed fatty acid. These data do not tally with the previous report<sup>1</sup>. The presence of a small amount of number of odd carbon chain fatty acids in the oil is also worth mentioning.

TABLE I  
Component acids of Kusum seed oil

Component acids	Percentage by weight
C <sub>14</sub>	0.4
C <sub>14</sub> :1	Trace
C <sub>15</sub>	0.1
C <sub>15</sub> :1	Trace
C <sub>16</sub>	16.5
C <sub>16</sub> :1	0.4
C <sub>17</sub>	0.2
C <sub>17</sub> :1	0.2
C <sub>18</sub>	6.4
C <sub>18</sub> :1	22.0
C <sub>18</sub> :2	49.6
C <sub>11</sub> :3	2.2
C <sub>20</sub>	1.2
C <sub>22</sub>	0.6
C <sub>23</sub>	0.2

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#### A NOTE ON THE BARYTE OCCURRENCES IN RALAM-GARBYANG SEQUENCE OF KUMAUN HIMALAYA

To the north of the Central Crystalline Zone of the Kumaun Himalaya a thick sequence of Pre-Precambrian-Palaeozoic-Mesozoic sediments is exposed. The lower part of the sequence comprises the Martoli, the Ralam and the Garbyang metasediments and sediments<sup>1-2</sup>. Within the lower Garbyang of the Girthi-Ganga valley of Chamoli District a zone bearing baryte mineralization has been discovered during the 3rd Himalayan Expedition sponsored by the Wadia Institute of Himalayan Geology.

Baryte occurs in thick veins associated with the dolomitic limestone of Lower Garbyang Series. The veins range in thickness from a few centi-

metres to 80 cm and persist for over 12 metres without pinching before they get covered by rock debris. The general trend of the veins is NW-SE to NNW-SSE. Some of the best exposures of these veins are found along the nearly vertical rock face of Girithi-Ganga gorge in the road cutting near Barmatiya (16 km from Malari along road). In addition disseminations and veinlets of chalcopyrite, bornite and their oxidation products have also been observed. A number of old workings of copper dot the area from about 12 to 17 km from Malari along mule track towards Sumna.

It is noted that this zone of mineralization is developed along the down-throw side of an important strike-fault which brings the Ralam conglomerates and quartzites in contact with the younger slates and dolomitic limestone of the Garbyang Series and this offers an interesting structural setting. The baryte and copper mineralization is restricted to the lower part of the Garbyang and has so far not been noticed in the Ralam Series that is exposed on the other side of the fault. The fault plays an important role in the localization of the mineralization.

The veins of baryte are white and massive and a specimen on analysis yielded 64.62% of BaO. The preliminary investigation reveals that this could be a promising deposit. Detailed work is in progress.

The authors are grateful to Professor A. G. Jhingran for encouragement and valuable suggestions and to Dr. K. S. Valdiya for useful comments. Sincere thanks are due to other members of the Expedition, Drs. V. K. Gairola and S. Kumar for discussions.

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#### ON THE OCCURRENCE OF "BLOCK STRUCTURES" IN THE ANORTHOSES AROUND ODDANCHATRAM, MADURAI DISTRICT, TAMIL NADU

THERE are good exposures of anorthosites in Kulandeivelappan hill (1381) to the west of Oddanchatram in Palni Taluk, Madurai District. In some outcrops of this locality, lens-shaped or polygonal blocks of coarse grained, brownish gabbroic anorthosite are found enclosed in massive coarse grained white anorthosite (Fig. 1). In some

places, the two are separated by an intervening layer of medium grained equigranular gabbroic rock. The contacts are either sharp or gradational. In some blocks, the mafic constituents show a dimensional alignment giving rise to a lineation. There are also inclusions of white anorthosite blocks in gabbroic rocks in a few outcrops (Fig. 2). The size of the blocks is within four meters.

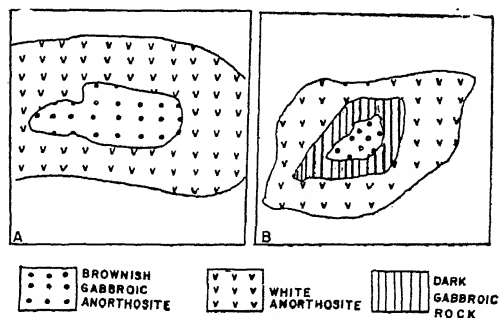


FIG. 1. Two sketches showing the block structures.

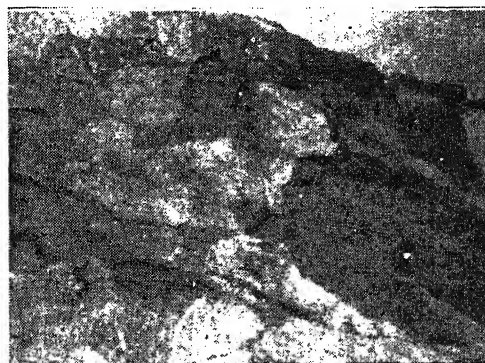


FIG. 2

Balk (1931) refers to the occurrence of inclusions of anorthosite in anorthosite in the Adirondack region as "Block Structures". Phinney (1969) describes also inclusions of anorthosite in a variety of dikes and mafic rocks in Keweenaw, NE Minnesota.

In the area under discussion, the anorthositic series of rocks comprise anorthosite, gabbroic and noritic anorthosite, gabbro, norite and minor pyroxene-ore rock. The anorthosites and gabbroic anorthosites are generally coarse grained with megacrysts of grey, pale brown or pale pink plagioclase set in a comparatively finer grained matrix of dull white plagioclase. The gabbroic rocks are fine to medium grained, melanocratic and equigranular. The plagioclase megacrysts in some places display a linear disposition revealing a semblance of primary flowage structures. The



following are the average modal compositions (Vol. %) of the important rock types of the series :

	Anorthosite	Gabbroic anorthosite	Gabbro
Plagioclase	.. 96.5	80.7	42.8
Potash felspar	.. ..	2.4	4.3
Hornblende	.. 2.4	9.2	9.0
Hypersthene	.. ..	3.1	6.8
Augite	.. ..	4.4	30.9
Others (ore, sphene)	1.1	0.2	6.2

Modal plagioclase  $Ab_{44}An_{56}Ab_{40}An_{60}Ab_{43}An_{57}$

Detailed field and laboratory investigations by the author have revealed that the anorthositic rocks of the area belong to massif type. They constitute a labradorite type pluton emplaced as a phacolith in an NNE plunging anticlinal fold structure of metasediments, basic granulites and charnockites. A domical form is indicated for the mass by the outward dipping foliation all around the pluton.

In the case of block structures observed in Kulandeivelappan hill, the host is more leucocratic than the blocks, in general. There is no significant difference in the An content of the plagioclase of the inclusion and the host rock. The host rock being more leucocratic than the inclusion appears to be somewhat exceptional from the generally observed relation. The occurrence of such reverse relations in some places in the Adirondack anorthosites has been recorded by Buddington (1939). The block structure in the area which is developed as a small scale feature is confined to the border facies as in the case of Adirondack anorthosites. The host rock is generally structureless while the blocks show in some places a lineation. The distribution of the rock types of the anorthositic series reveals that the anorthosites occupy the central portions of the mass while the gabbroic anorthosites and gabbros tend to be more segregated towards the margins.

The field relations do not indicate the possibility of the development of the block structures by any successive upsurges of different magma types. It is reasonable to suggest that they resulted from the flow differentiation of the parent gabbroic anorthosite magma which caused a lateral segregation of different rock facies of the series as advocated by Buddington (1969). The lineated character of some of the blocks is also considered as a primary

feature as visualised by Davis (1969) for the block Structures of St. Regis Quadrangle, New York.

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### EFFECT OF SOIL SALINITY ON THE VIABILITY OF WHEAT SEEDS

It has been observed that percentage germination of wheat seeds is less when seeds are collected from the Soil Salinity Institute Farm as compared to seeds collected from outside the farm having normal soil. A laboratory experiment was, therefore, conducted to test the viability of wheat seeds collected from wheat grown on saline soil (Silty clay loam, pH 5.8, E.C.—9.0 m.mhos/cm, ESP 20.0) as well as seeds collected from normal soil (Loam, pH—6.0, E.C.—0.5 m.mhos/cm).

Wheat seeds (var. Kalyan Sona) were collected one month after the harvest of the crop and germination percentage was studied from May to October, 1973 in petridish with three replications and with 100 seeds in each replication. Results are presented in Fig. 1.

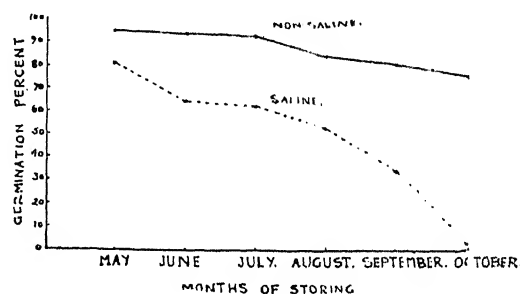


FIG. 1. Viability of wheat seeds collected from saline and non-saline soils.

Figure 1 shows that germination percentage of wheat seeds throughout the period of study, collected from normal soil, was more than that collected from saline soil. Viability of seeds did not decrease up to July. However, thereafter there was a slow decrease in case of the seeds collected

from the normal soil, whereas viability of the seeds collected from saline soil area decreased sharply even after May. Later on decrease was gradual up to September and in October viability of the seeds was practically lost (1% germination). Moisture percentage of both the seeds was determined and it was found to be the same (18% on dry weight basis).

Sugar content of the seeds from October samples was estimated by Somogy's method and the results are presented in Table I.

TABLE I

*Sugar content of wheat seeds six months after storing*

	Reducing Sugar %	Total Sugar %
Seeds collected from saline soil field ..	0.016	2.07
Seeds collected from normal soil field ..	0.010	4.40

It is seen from Table I, that total sugar content of seeds collected from wheat crop grown on saline soil is less than that of the seeds collected from the crop grown on normal soil, whereas reducing sugar content is the same in both the cases. Thus soil salinity decreases the total sugar content of the wheat seeds and thereby affects their viability.

Thanks are due to Dr. A. K. Bandopadhyaya, Soil Physicist, for his keen interest and helpful guidance. Thanks are also due to Shri T. S. Sinha for his valuable suggestions in this investigation.

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#### ROLE OF ACTINOMYCETES IN THE BIO-SYNTHESIS OF INDOLE ACETIC ACID IN SOIL

AUXINS are present in soil, synthesized by the autochthonous microflora as they decompose carbonaceous matter<sup>1,2</sup>. Indole acetic acid (IAA) and other auxin-like substances, gibberellins and gibberellin-like compounds have been isolated or their activities found in culture filtrates of a high percentage of heterotrophic microflora dwelling in environments where they might influence plant growth<sup>3,4</sup>. In an earlier communication from this laboratory<sup>5</sup> it was brought out that the soil micro-organisms are potential synthesizers of indole auxins (IAA) provided suitable precursors are available in soil in adequate quantities, however, the role of soil actinomycetes in this process has not been

precisely understood. In the present communication the participation of soil actinomycetes in the build up of IAA-pool in soil is reported.

From the soils of the Experimental Farm, Tamil Nadu Agricultural University, Coimbatore, several actinomycete cultures were isolated using Küsters agar medium<sup>6</sup>. Fifteen of them were selected and screened for their *in vitro* production of IAA. The isolates were grown in 250 ml Erlenmeyer flasks containing 50 ml of Czapek's medium supplemented with 0.005 M DL-tryptophan (L-amino-3-indole propionic acid). The flasks were incubated at 25° C in darkness for 15 days under static conditions. IAA was determined quantitatively in the clear cell-free culture filtrate obtained through 'Seitz' filtration. Salper's reagent was used to develop colour in the samples<sup>7</sup>. The colour development was allowed to proceed exactly for 1 hr in darkness and the colour intensity was read in a Spectronic-20 colorimeter at 535 mμ. The results are presented in Table I. The promising actinomycete cultures (Sm. 9 and Sm. 10) which synthesized appreciable quantities of IAA were studied further in sterile and unsterile soils.

TABLE I

*Synthesis of IAA by different isolates of actinomycetes\**

Isolates	IAA synthesized (μg/100 ml of the culture filtrate)
Sm. 1	236.39
Sm. 2	85.96
Sm. 3	113.59
Sm. 4	110.52
Sm. 5	141.22
Sm. 6	291.65
Sm. 7	0.67
Sm. 8	0
Sm. 9	1181.95
Sm. 10	1135.08
Sm. 11	61.40
Sm. 12	150.43
Sm. 13	114.29
Sm. 14	0
Sm. 15	92.10

\* Data represent average of three estimations.

Fifty g of the well-sieved red soil was taken in 500 ml Erlenmeyer flasks. While one set of such flasks was sterilized in an autoclave, another set remained unsterile. An aliquot of 50 ml of 0.05 M phosphate buffer (pH 7.0), 0.005 M DL-tryptophan and 10 g of dextrose were added to the flasks. The flasks were then inoculated with 10 ml spore suspension of the actinomycete isolate (Ca. 10<sup>8</sup> cells/ml), mixed thoroughly and incubated in darkness at room temperature (28 ± 1° C) for 24 hr. The spore suspensions

were collected from 15 days old growth of the isolates on Kuster's agar petri plates. Sample controls and replications were maintained. The clear soil extract was obtained by centrifugation at 2,100  $\times$  g for 15 min in an International centrifuge. IAA contained in the soil extract was determined quantitatively<sup>8</sup>. For identifying IAA in the culture filtrate as well as in soil extracts, the condensed final extract was spotted on silica gel G coated thin layer chromatographic (TLC) plate and developed in *iso*propanol:ammonia:water (10:1:1 v/v/v) solvent system and Salkowski reagent for locating the IAA (R<sub>F</sub> 0.58) on the chromatogram<sup>9</sup>.

TABLE II

IAA synthesis in sterile and unsterile soil by actinomycete isolates

No.	Treatment	Quantity of IAA synthesized ( $\mu$ g/25 ml of soil extract)	
		Sterile soil	Unsterile soil
1.	Soil (Control)	0	0
2.	Soil + dextrose	0	12.340
3.	Soil + dextrose + tryptophan	0	138.640
4.	Soil + actinomycete, Sm. 9 + dextrose	12.720	28.620
5.	Soil + actinomycete, Sm. 10 + dextrose	8.680	24.840
6.	Soil + actinomycete, Sm. 9 + dextrose + tryptophan	64.935	172.260
7.	Soil + actinomycetes Sm. 10 + dextrose + tryptophan	48.220	153.660

\*Data represent average of three estimations.

The results in Table I indicate that of the 15 actinomycete cultures tested, with the exception of two, all synthesized IAA in the culture medium. The isolates Sm. 9, and Sm. 10 were efficient in this respect. When the two isolates were incorporated in sterile and unsterile soils as spore suspensions, there was no appreciable increase in the IAA level in sterile soil compared to that in unsterile soil. This might be due to the zymogenous soil population which would have contributed to the IAA status of soil<sup>2</sup>. Of further interest was that when the soils were supplemented with tryptophan, the precursor of IAA, the recovery of IAA was more in the two soils. Here again the unsterilized soil recorded a greater IAA 'turn-over' than the sterile soil. The possible explanation would be that in unsterile soil, in addition to the inoculated actinomycete cultures, various groups

of micro-organisms were present in the soil. The recovery of IAA in the soil was not only dependent on the soil type but also on the type of micro-organisms present in the soil. The recovery of IAA in the soil was not only dependent on the soil type but also on the type of micro-organisms present in the soil.

The authors are grateful to Dr. C. D. G. Ramakrishnan, Vice-Chancellor, Tamil Nadu Agricultural University, Coimbatore for his helpful suggestions and facilities. The authors are also grateful to Dr. P. K. Mahadevan, Land Naids, Agricultural University, Coimbatore for his helpful suggestions and facilities. The authors are also grateful to Dr. P. K. Mahadevan, Land Naids, Agricultural University, Coimbatore for his helpful suggestions and facilities.

The authors are grateful to Dr. C. D. G. Ramakrishnan, Vice-Chancellor, Tamil Nadu Agricultural University, Coimbatore for his helpful suggestions and facilities. The authors are also grateful to Dr. P. K. Mahadevan, Land Naids, Agricultural University, Coimbatore for his helpful suggestions and facilities.

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**EFFECT OF POTASSIUM AND ZINC IONS ON  
GROWTH AND TRYPTOPHAN SYNTHETASE IN  
ALKALOID PRODUCING CULTURE OF  
*ASPERGILLUS FUMIGATUS***

TRYPTOPHAN is known to be a precursor of ergot alkaloids in saprophytic *Claviceps* species<sup>1-3</sup>. In our earlier study<sup>4</sup> while establishing nutritional requirements for the production of alkaloids by *Aspergillus fumigatus* we have observed that

prepared and assayed according to the method of Nason *et al.*<sup>7</sup>. For endogenous tryptophan the dry mycelial pads were ground to a fine powder with a mortar and pestle. A 2 ml amount of boiling water was added to 100 mg of the powdered mycelium and the resulting suspension was centrifuged at 15,000 xg for 15 min; the supernatant was filtered and tryptophan was measured by the method of Spiess and Chambers<sup>8</sup>.

TABLE I

*Effect of potassium and zinc concentrations on growth, alkaloid formation and tryptophan synthetase of A. fumigatus\*\**

K/Zn	Dry mycelium gm/litre	Alkaloids mg/litre	Free tryptophan mg/g dry mycelium	Tryptophan synthetase units/mg protein
(*Potassium mg/litre)				
0.0	3.8	10.0	0.7	0.8
50.0	15.0	46.0	1.5	1.2
100.0	20.0	66.0	1.8	1.6
150.0	26.0	80.0	2.0	2.0
200.0	24.0	112.0	3.0	3.6
250.0	23.5	80.0	1.9	1.8
(*Zinc mg/litre)				
0.0	4.0	10.0	0.6	0.6
0.25	13.5	22.0	1.4	1.2
0.50	25.0	70.0	2.0	2.0
0.75	21.0	110.0	3.1	4.0
1.00	20.0	80.0	2.4	2.2
1.25	17.5	70.0	1.9	2.0

\* K and Zn were supplied in terms of cations and demineralized water was used for medium.

\*\* In 96 hrs old culture.

potassium and zinc (when supplemented to the medium as sulphates) play a prominent role on growth as well as alkaloid production. There are reports<sup>5,6</sup> in literature indicating that minerals are essential for alkaloid production, however, there is no report directly correlating the role of minerals in alkaloid formation. In this paper, we wish to report that potassium and zinc stimulates alkaloid formation, by supplying more tryptophan through increased activity of tryptophan synthetase.

The strain of *A. fumigatus*, the medium composition, and the procedures for extraction and estimation of alkaloids used in the present investigation were similar to those described earlier<sup>4</sup>. Cell-free extract of tryptophan synthetase was

The results presented in Table I show that the omission of potassium and zinc from the culture medium caused a decrease in both growth and alkaloid production. Alkaloid production reached maximum (112 mg/litre) when the potassium content of the medium was 200 mg/litre. At that concentration of potassium, there is more (four fold) free endogenous tryptophan available to the culture due to the increased activity of tryptophan synthetase by four fold. Similarly 0.75 mg of zinc per litre gave 110 mg of alkaloids. Here also the increase in alkaloid yield may be due to the availability of more tryptophan (3.1 mg/g dry mycelium) in comparison to control. Further, when tryptophan is initially supplemented (100 mg/litre)

to the cultures of 50 mg/litre of potassium and 0.25 mg/litre of zinc, they also gave yield approximately 100 mg of alkaloids per litre. The above results clearly indicate that either absence or limited concentration of potassium and zinc decrease the synthesis of tryptophan ultimately reducing the yield of alkaloids. Tryptophan synthetase activity is considerably inhibited in cell-free extracts of potassium and zinc deficient *Aspergillus fumigatus*. This is in line with the reports of Nason *et al.*<sup>7</sup> in *Neurospora crassa* and Schwartz and Bonner<sup>8</sup> in *Bacillus subtilis*. The role played by tryptophan in alkaloid synthesis becomes significant when one considers that the major portion of the ergoline nucleus of the alkaloids is derived from tryptophan. In *A. fumigatus* also our earlier results<sup>4</sup> indicated that tryptophan do stimulate alkaloid formation.

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#### IDENTIFICATION OF GRASSY STUNT, A NEW VIRUS DISEASE OF RICE IN INDIA

THE *Mundakan* and *Punja* rice crops of Trichur and Kuttanad in Kerala State were severely damaged by an outbreak of brown plant hopper and an unidentified virus disease during 1973-74. This virus disease was responsible for heavy yield losses of *Punja* crop in Kole area of Trichur District and Upper Kuttanad area of Alleppey District. The estimated percentage of infection at the time of observation (26th January to 1st February, 1974)

varied between 30 to 90% in transplanted cultivars *IR 8* and *Jaya* and 5 to 15% in direct sown cultivar *Triveni* in Trichur District and 70 to 80% in *Jaya* in Upper Kuttanad of Alleppey District. Approximately 15,000 hectares were affected.

Severely infested fields appeared rusty brown or yellow in colour at a distance. On a close observation the plants were severely stunted and bore excessive number of tillers. The growth habit of the plants was erect. The leaves were short, narrow, erect and pale green or yellow. Young leaves sometimes exhibited interveinal chlorotic mottling or stripes. Most of the old leaves had numerous characteristic dark brown spots of various dimensions. The diseased plants generally did not produce any panicles. Sometimes, a few worthless panicles with dark brown grains emerged from the diseased plants.

Transmission studies of typical infected plants were carried out at Central Rice Research Institute. Since this disease was associated with brown plant hopper, *Nilaparvata lugens* (Stål), transmission was attempted with this hopper. Twenty fairly grown nymphs or adults of the hopper were confined on the diseased plant for five days. Starting from the 6th day, the individual hoppers were transferred to fresh set of 20-day-old seedlings every day until the 26th day. Five hoppers were alive until the last day. Ten hoppers became viruliferous and transmitted the disease in total to 45 plants. The incubation period ranged from 10 to 18 days in the vector and 6 to 10 days in the plant. The viruliferous hoppers after incubation period could transmit the disease until their death. These preliminary results demonstrated the persistent nature of the virus.

Symptoms and transmission properties clearly indicate that this disease is identical with grassy stunt virus disease reported by Rivera *et al.*<sup>2</sup> from the Philippines, and may be taken as the first authentic report of the disease from India.

Suspected grassy stunt diseased plants were earlier observed from India<sup>2</sup>. Das *et al.*<sup>1</sup> reported a virus disease approximately resembling grassy stunt transmitted by *Nilaparvata lugens* from Sambalpur, Orissa. They pointed out some symptomatological differences between the disease and grassy stunt and thus posed a doubt. Repeated transmission tests from the diseased plants collected from Mr. S. R. Das, Sambalpur, conducted by the author with *Nilaparvata lugens* at Central Rice Research Institute were unsuccessful.

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#### A NEW GRAFT-TRANSMISSIBLE DISEASE OF COWPEA [*VIGNA SINENSIS* (TORNER) SAVI]

COWPEA, an important pulse crop, is infected by a number of virus diseases (Nene, 1972). During the survey for the virus diseases, cowpea plants were observed to show smalling of leaves and malformations of floral parts, in addition to stunting of plants indicating the involvement of a new causative agent. Studies on the transmission and symptomatology were taken up to elucidate the nature of the causal agent of this disease which has not been reported earlier on this host.

Scions from naturally infected plants were side-grafted to 25 days old cowpea plants. The cowpea varieties K 11, PS 42, Co 2 and C 152 were used as test plants.

TABLE I

Graft transmissibility of cowpea phyllody disease

Varieties	Number of plants grafted	Number of plants infected
K 11 ..	2	1
PS 42 ...	6	4
CO 2 ..	3	2
C 152 ..	4	2

While the variety CO 2 took about 60 days for the manifestation of the symptom, the varieties C 152, K 11 and PS 42 produced symptoms after about 45 days. After grafting, the top of the rootstock was clipped off and the side shoots produced subsequently were also topped and then the side shoots produced typical symptom.

Attempts to transmit the disease through sap inoculation were not successful. The transmission experiments using leafhoppers, white flies and aphids are in progress.

The disease was characterised by stunting and smalling of leaves in the initial stages (Fig. 1). Later when the plants reached flowering stage, the floral parts were transformed into green, leaf-like

structures followed by abundant vegetative growth. The five sepals became leaf-like and were unusually big in size. The corolla was either partially green or turned into papery structures. The veins of the petals and sepals were thickened and appeared prominently (Fig. 2).

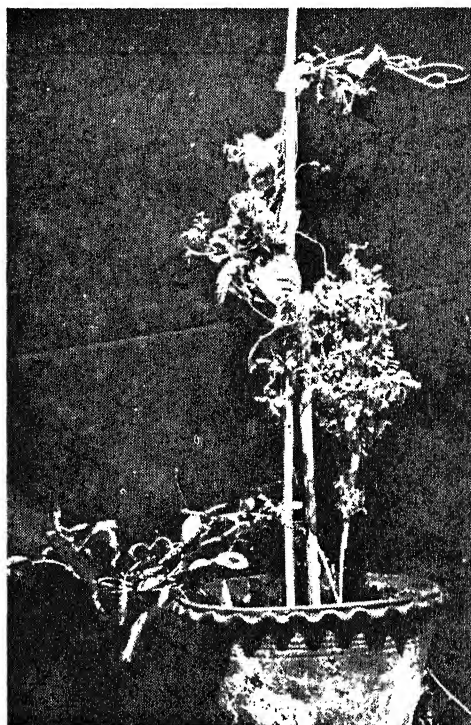


FIG. 1. Infected cowpea plant showing smalling of leaves and mosaic patterns.

In the place of the ovules, there were small petiole-like outgrowths which later grew and burst through the wall of the false ovary producing small shoots. These shoots continued to grow and produced more leaves and phyllid flowers. The normal flowers have short pedicels whereas the stalk of a phyllid flower is very much elongated.

The stamens generally retained their normal shape but they were thin and papery. The anthers were slender and contained a few viable pollen grains. In most cases, the flowers did not produce any fruits. But in some cases, the plants produced thin, dwarf, slender and papery pods. The leaves of infected plants showed mosaic patterns. The size of the leaves in the terminal shoots was considerably reduced and the internodes were shortened.

Production of new shoots from closely placed axils due to possible stimulation of axillary buds resulted in crowding of shoots at apical portions, giving a bushy appearance to the plants. Based

on these symptoms the disease is named as cowpea phyllody disease.



FIG. 2. Phyllod nature of flowers.

The similarity of symptoms is suggestive of possible relationship with the causative agent of phyllody disease of gingelly (Vasudeva and Sahambi, 1955). However, further studies are needed to establish such a relationship.

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#### THE EFFECT OF DIFFERENT CITRUS VIRUSES ON PHOTOSYNTHETIC PIGMENTS

THE following virus diseases (1) mosaic, (2) infectious variegation (Reddy *et al.*, 1972)<sup>1</sup>, and (3) yellow corky vein<sup>2</sup> were recently reported from certain Citrus growing areas of Andhra Pradesh. Another disease commonly known as *Sankhu*

*tegulu* (unidentified) is quite predominant in some of the areas and resembles to a large extent the greening disease. A very common resultant effect of these virus infections was found to be the yellowing of the entire leaf or certain areas of the leaf. Hence, a study has been conducted to evaluate the effect of different viruses on the amounts of photosynthetic pigments, chlorophyll *a* and chlorophyll *b*. Though there are general reports of certain viruses decreasing the chlorophyll content (Diener, 1963)<sup>3</sup>, no specific study seems to have been made in the above-mentioned viruses on Citrus.

Except for infectious variegation where Lisbon lemon formed the host, Sathgudi plants were used as the host for the remaining viruses. The material for *Sankhu tegulu* (unidentified) was collected from an orchard near Kadiri (Andhra Pradesh). One to two year old Sathgudi and Lisbon lemon plants were inoculated with the different viruses in November 1972 and the estimations were carried out in July 1973. Severity of the symptom expression was taken into consideration and keeping leaf position constant, the material was collected from both healthy and diseased plants of the same age.

Chlorophyll *a* and *b* were estimated following the method of Arnon (1949)<sup>4</sup>. Leaf material equivalent to 200 mg fresh weight was extracted with 80% acetone and filtered. The filtrate was made upto 25 ml and the optical density of the extract was read at 645 and 663 nm for chlorophyll *a* and chlorophyll *b* respectively, and the results are presented in Table I. The values given in the table are the mean of three replications. In addition to chlorophyll *a* and chlorophyll *b*, total chlorophyll, 'a/b' ratio as also per cent reduction are included in the table.

It could be seen from Table I that in all the four diseases under study there is a general reduction in chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in the diseased leaves when compared to the healthy ones. The maximum reduction in total chlorophyll content (88.6%) is evident in plants affected by *Sankhu tegulu* (unidentified) and it is minimum in those affected by yellow corky vein (8.5%). On the other hand mosaic infection brought about a 56% reduction, while infectious variegation resulted in 15% reduction in the total chlorophyll content. Except for slight variations, the percentage reduction in chlorophyll *a* and chlorophyll *b* are the same as that of the total chlorophyll. Virus infections [excluding *Sankhu tegulu* (unidentified)] seem to favour a slight rise in *a/b* ratio, while *Sankhu tegulu* resulted in a slight fall in the *a/b* ratio.

TABLE I  
Changes in chlorophyll 'a' and 'b' and total chlorophyll  
mg/g Fresh Weight

No.	Virus	Host		Chloro- phyll 'a'	Chloro- phyll 'b'	Total chlorophyll	Chl. 'a' Chl. 'b' ratio
1.	Mosaic	Sathgudi	Healthy	0.4291	0.1824	0.6115	2.357
			Diseased	0.1995	0.0702	0.2697	2.843
			% reduction	53.0	51.0	56.0	
2.	Yellow Corky Vein	"	Healthy	0.4544	0.1504	0.6048	3.023
			Diseased	0.4166	0.1358	0.5524	3.068
			% reduction	8.3	7.6	8.5	
3.	'Sankhu tegulu' (unidentified)	"	Healthy	0.3787	0.1759	0.5546	2.153
			Diseased	0.0421	0.0211	0.0632	1.998
			% reduction	88.8	88.0	88.6	
4.	Infectious variegation	Lisbon lemon	Healthy	0.1515	0.0789	0.2304	1.920
			Diseased	0.1296	0.0662	0.1958	1.958
			% reduction	13.7	16.1	15.0	

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**EPHESTIA CAUTELLA (WALKER) (PHYCITIDAE :  
LEPIDOPTERA) INFESTING STORED GARLIC  
(ALLIUM SATIVUM)**

In August, 1972, heavy infestation of *Ephestia cautella* was observed on garlic stored in a basket at Solan (1500 m.a.s.l.), Himachal Pradesh. This garlic was stored in May, after proper drying. A review of literature revealed that some lepidopterous larvae infested stored garlic in Hawaii and Philippines as early as 1915 (Banaag *et al.*, 1961; Ehrhorn, 1917 and 1926; Maskew, 1915 and 1918).

However, from India so far there is no record of *E. cautella* infesting garlic in stores, as far as the authors know. In the present investigations it was observed that the larvae generally started boring the bulb from the base and continued feeding inside the bulblets. The outer skin of the bulblet was found intact while the whole bulb from inside was consumed. Yellowish brown excreta was also seen near the point of entrance. The newly hatched larvae were offered bulblets, pricked at a number of places, for feeding. The larvae were seen entering the holes thus made and also fed inside. After two days excreta was seen coming out of the holes thereby indicating that the larvae were feeding



inside the bulblets. In heavy infestation the whole bulbs/bulblets were consumed (Figs. 1 and 2).

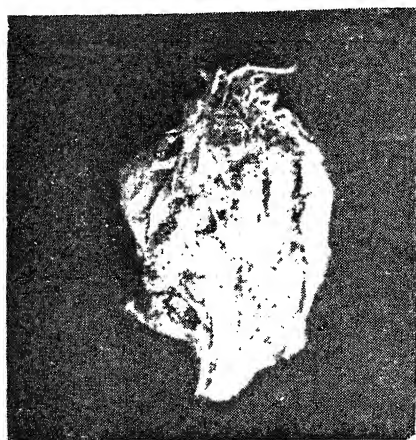


FIG. 1. Damaged garlic bulb.



FIG. 2. A. Damaged bulblet. B. Healthy bulblet.

A culture of *E. cautella* was maintained in the laboratory at  $27 \pm 1^\circ\text{C}$  in an incubator, throughout the year. It was found that this pest required about one month for completing one generation. At room temperature the insects entered hibernation during October and adults were seen emerging from the hibernating pupae during next April.

The identification of the insect was confirmed by the Commonwealth Institute of Entomology, London.

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#### ON THE FORM OF GILLRAKER SERRAE IN THE INDIAN *ILISHA*

WHITEHEAD (1967)<sup>1</sup> described the form of gillraker serrae as a distinguishing character in the species of *Setipinna*. The form of gillraker serrae in the Indian species of *Ilisha* is described in the present note. A slight but recognisable difference is recorded in different species. The genus *Ilisha* is under review by the author and six species are recognised from the Indian waters, viz., *Ilisha megaloptera* (Swainson)<sup>2</sup>, *I. sirishai* Seshagiri Rao<sup>3</sup>, *I. filigera* (Valenciennes)<sup>2</sup>, *I. elongata* (Bennett)<sup>2</sup>, *I. whiteheadi* Seshagiri Rao<sup>4</sup> and *I. melastoma* (Schneider)<sup>5</sup>. The form of gillraker serrae in these species has not been described so far.

Examination of gillrakers in the six species of *Ilisha* revealed that the serrae do not form distinct clumps as in *Setipinna taty* or *S. phasa*<sup>1</sup>. However the size and arrangement of serrae vary in the different species of *Ilisha*. In *I. megaloptera* the serrae on the upper surface of the gillraker are relatively larger, less numerous and crowded into groups with distinct gaps. Very few serrae are present on flanks. In *I. sirishai* the serrae are relatively smaller, numerous, uniformly distributed over the upper surface, descending on to the upper 1/4 of the flanks. In *I. filigera* the serrae are present in two or three rows on the upper surface with a few larger serrae towards the tip. A row of serrae is present on the lower flank of the gillraker. In *I. elongata* the serrae are more

numerous, distributed all over the surface of the gillraker with a few larger serrae towards the tip. In *I. whiteheadi* the serrae are smaller and sparsely distributed over the upper surface and upper 2/3 on flanks. In *I. melastoma* the gillraker serrae are very few, distributed in small groups over the upper surface and upper 1/4 on flanks.

The gillraker serrae in *I. megaloptera* are relatively larger in size, whereas in *I. melastoma* they are smaller. In *I. elongata* numerous serrae are arranged all over the surface of gillraker, whereas in *I. melastoma* their number is too small.

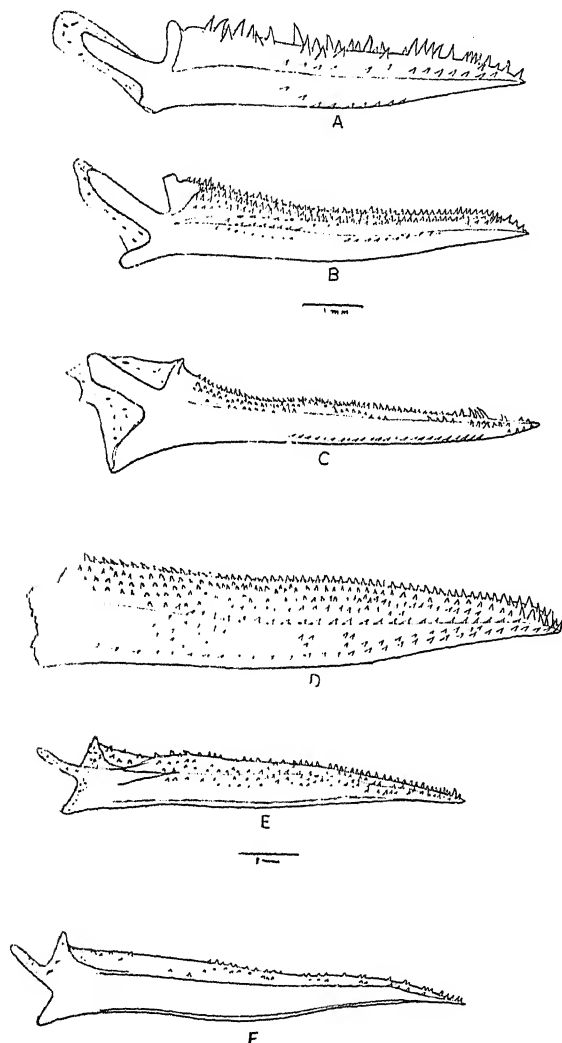


FIG. 1. Gillrakers showing the relative size and arrangement of serrae in *I. megaloptera* (A); *I. sirishai* (B); *I. filigera* (C); *I. elongata* (D); *I. whiteheadi* (E); and *I. melastoma* (F); (first gill arch, 4-5 gillraker on the lower arm from the angle).

The author is grateful to Professor S. Dutt, Guntur and P. J. P. Whitehead, British Museum (Natural History), London, for encouragement. Thanks are due to N. Bangar Raju, Principal, for encouragement and to the University Grants Commission, New Delhi, for financial assistance.

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#### OCCURRENCE OF AN ACCESSORY HAEMO- CHORIAL PLACENTA IN THE INDIAN LEAF-NOSED BAT, *HIPPOSIDEROS FULVUS FULVUS* (GRAY)

OUR knowledge concerning the embryology of *Hipposideridae* (Microchiroptera) is limited to a few stages of development of only two species—*Hipposideros bicolor pallidus* (Gopalakrishna, 1958; Gopalakrishna and Moghe, 1960), and *Hipposideros fulvus fulvus* (Karim, 1972). In both the species the placenta at an advanced stage of gestation has been described as being double discoidal, mesometrial and vasochoial. The ripe placenta has, however, not been described in either of the species.

While making a detailed examination of the ripe placenta of *Hipposideros fulvus fulvus* the present author noticed the occurrence of an unusual conical structure (Fig. 1) lying between the placental discs. Since this structure is not noticed during the earlier stages of gestation it is evident that this structure develops only during the final stages of pregnancy. Examination of serial sections of the ripe placenta revealed that this conical structure is actually an accessory placenta, which differs from the main placental discs in histological details. The main placenta of this species is vasochoial during the final stages of pregnancy but the accessory placental cone presents several interesting features. In sections stained in haematoxylin-eosin (Fig. 2) the accessory placenta appears to be composed of numerous tortuous tubules between which lie foetal mesenchyme and foetal blood capillaries. The wall of the placental tubules is made up of a mass of eosinophilic cytoplasm in which broken bits of nuclei and chromatin lie scattered haphazardly. It is not possible definitely to state if the cytoplasm belongs exclusively to the trophoblast or it is a mixture of the cytoplasm of trophoblast and endothelium

since there is no demarcation between the two. Organized nuclei are very rare in this cytoplasmic mass, and those present are highly pycnotic indicating that they are on their way to destruction. Maternal blood corpuscles are noticed within the tubules. In sections stained by PAS procedure (Fig. 3) a thin, but distinct, scarlet coloured homo-

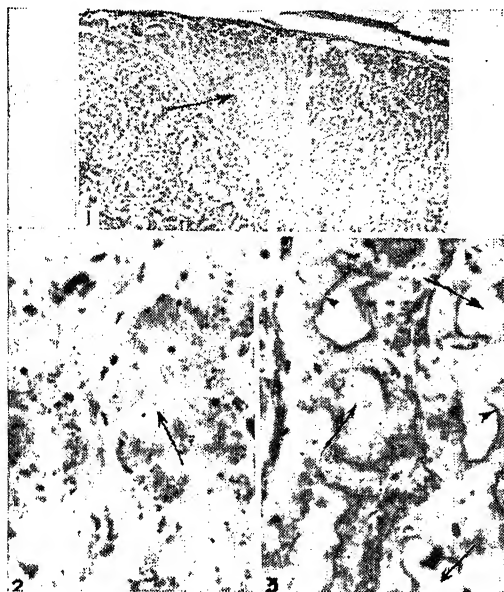
I am grateful to Prof. Dr. A. Gopalakrishna, D.Sc., Director, Institute of Science, Nagpur, for encouragement and guidance in this study.

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FIGS. 1-3. Fig. 1. Part of the ripe placenta of *Hipposideros fulvus fulvus* to show the accessory placenta (arrow) between the bases of the two main placental discs.  $\times 40$ . Fig. 2. Two placental tubules of the accessory placenta (Haematoxylin-eosin). Note the presence of nuclear fragments and pycnotic nuclei in the wall of the tubules. Arrows indicate the lumen of the placental tubules.  $\times 325$ . Fig. 3. Two tortuous placental tubules (PAS-Weigert's haematoxylin) to show the interstitial membrane (arrow heads) and the lumina of the tubules (arrows). Also note the presence of nuclear fragments and pycnotic nuclei in the wall of the tubules.  $\times 325$ .

geneous, nonfibrous membrane is noticed embedded in about the middle of the thickness of the wall of the tubules. This is apparently the remnant of the "interstitial membrane" (Wimsatt, 1958) of the placental tubules and is, in many places, discontinuous. Hence, there could be a free percolation of the cytoplasm between the trophoblastic syncytium on the foetal side and the endothelial cells on the maternal side of the interstitial membrane. Evidently, this is a very special type of haemochorial relationship which has not been so far described in any mammal. The significance of the breakdown of the nuclei of the trophoblast and that of the endothelium is not clear.

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#### EFFECT OF SIMULATED RAIN ON THE SURVIVAL OF FIRST INSTAR LARVAE OF *CHILO PARTELLUS* (SWINHOE), CHAMBIDAE : LEPIDOPTERA

DURING the course of studies on the natural population of *Chilo partellus*<sup>1</sup>, a serious pest of maize and sorghum, it was observed that the proportion of younger larvae (first-second instar) in the larval population was relatively less after a rainfall than that observed during other periods. It was believed that rainfall was an important abiotic factor for the survival of younger larvae.

The effect of simulated rain on survival of the first instar larvae of *C. partellus* was studied in the laboratory. The first instar larvae, just after hatching, were released in the whorls of 10-day old potted plants of maize. They were allowed to feed and settle in the whorl for 24 hours before the experiment. Rain was simulated by using a rocking spray pump fitted with Hyjet gun (of M/s. American Spring and Pressing Works, Private Limited, Bombay), which was calibrated earlier. For getting one centimeter of rain, water was sprayed under a constant pressure of 7.03 kg/cm<sup>2</sup> for 11 minutes about 25 cm above the potted plants. The spraying time was increased to 22 minutes for getting a rainfall of 2 cm and to 33 minutes for 3 cm, under the same constant pressure. The velocity of wind ranged from 0 to 1 km per hour during the experiment. A control was also kept to know the mortality among the larvae without rain. Mortality counts were made after 24 hours of treatment by dissecting the plants with the help of a grafting knife. The results are given in Table I.

It was observed that 17.8% of the larvae died with a rainfall of 1 cm. The larval mortality increased to 66.6% with 2 cm rainfall and to 85.8% with 3 cm rainfall. It is thus indicated that a heavy rainfall, more than two centimeters, during the period of activity of *Chilo partellus* would cause considerable mortality among its first instar larvae.

TABLE I

Effect of simulated rain on the survival of first instar larvae of *Chilo partellus*

Rain simulated (cm)	Number of larvae		per cent mortality	Mortality in control (%)	Corrected mortality (%)
	exposed	dead			
1	450	105	23.33	6.67	17.8
2	300	206	68.66	6.0	66.6
3	150	130	86.66	6.0	85.8

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#### AN INBRED LINE OF RADISH (*RAPHANUS SATIVUS* L. VAR. *RADICOLA* PERS.) WITH ABNORMAL MALE GAMETOPHYTE

ABOUT 40 inbred lines have been isolated from three varietal populations of radish, namely "Saxa", "Icecle" and "Virovsky bellie" by Dr. S. I. Narbut of the Chair of Genetics and Plant-breeding, Leningrad State University, Leningrad (U.S.S.R.) and are being maintained. These lines are characterized by reduced fertility and autofertility, low vigour in growth, reduced chiasma frequency, abnormal meiosis, etc., in comparison to their population plants<sup>1-5</sup>. There are several lines which show the development of genetic tumours on root and possess yellow green leaves. One of such lines is LS-337/24 ( $I_{12}$ ) which has been derived from the "Saxa" and is cytogenetically well studied<sup>1</sup>. The present report deals with the study of mature pollen grains of this line and the varietal population.

Mature flower buds were fixed in Carnoy's solution for 6 hours. The anthers were stained and squashed in 1% acetocarmine and examined under the microscope.

The mature pollen grain of radish is round in shape having a diameter of about 30  $\mu$ . The exine is thick and smooth. The division of the generative nucleus takes place within the pollen grain which is a trinucleate structure with two male gametes and one tube nucleus. In the population plants the mature pollen grains were trinucleate structure as described above. But in the plants of the inbred line, LS-337/24 ( $I_{12}$ ) some of the pollen grains,

though their number is insignificant and hardly exceeds 1%, showed the presence of three gametes besides the tube nucleus. This is a peculiar situation, which, to the best of the knowledge of the author, is unreported.

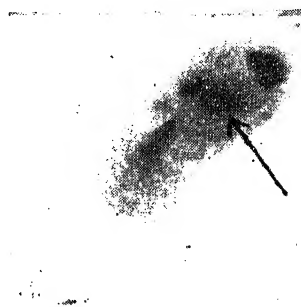


FIG. 1. A mature pollen grain of the inbred line LS/337/24 showing three male gametes.

It is well known that forced inbreeding breaks up the buffering property of allogamous populations as a result of which a large number of genotypes (lines), having different degree of segregation and combination of genes, arise. In such genotypes homozygosity is increased manifold<sup>3-4</sup>. Rees has proposed polygenic control of chromosome behaviour in rye on the basis of his experiments with inbred lines<sup>6</sup>. Gottschalk and Baquar have reported a number of genes responsible for normal chromosome behaviour in *Pisum*<sup>2</sup>. It has also been pointed out by them that some of these "meiotic genes" might affect the gametogenesis. In the present case some "meiotic gene or genes" seem to have affected the gametogenesis as a result of which more than two male gametes develop in the pollen grain. Besides this situation might arise due to the loss of "genetic homeostasis" in the plants of the inbred line under continued forced self-pollination.

The author expresses his sincere thanks to Prof. T. S. Fadeyeva and Dr. S. I. Narbut of the Chair of Genetics and Plant-breeding, Leningrad State University, Leningrad, for valuable suggestions and criticism.

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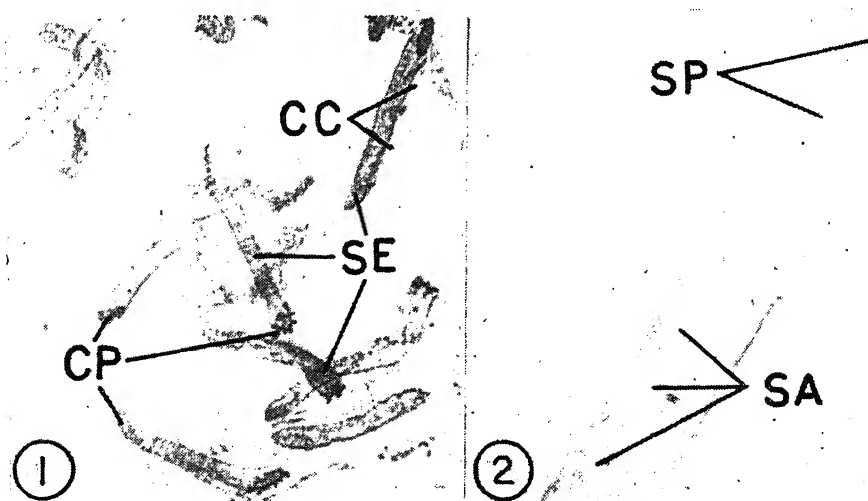
### A SIMPLE MACERATION TECHNIQUE FOR THE SEPARATION OF SIEVE ELEMENTS FROM THE BARKS OF WOODY PLANTS

OWING to its delicate nature and peculiar histochemical properties, the study of phloem requires more exacting techniques than xylem<sup>1</sup>. Leaving aside the fibres and sclereids the other elements of phloem, particularly those which are directly involved in the transportation process, namely, the sieve tube members, do not develop rigid, persisting walls as the xylem, and mostly remain delicate during their functional period (in the majority of the woody plants the functional period is not more than a few months' duration). Later, when the phloem ceases to act as a conducting tissue, the sieve elements become either generally disorganised or get modified to various degrees. Thus in the phloem, the conducting elements lose their original structure and appearance very early as compared to others. It, therefore, becomes necessary to investigate this tissue soon after its inception or before it ceases to become a non-conducting tissue. Due to these difficulties, proper techniques to investigate this delicate tissue did not come out in great number as it did for the study of xylem. As a consequence, our knowledge of this tissue still remains mostly incomplete, both regarding its structural variations and its phylogeny<sup>1</sup>, although the discovery of phloem, as a conducting tissue, dates back to the first half of the previous century<sup>2</sup>. In the present study an attempt has been made to evolve a simple technique to obtain the sieve elements in macerated condition to study their

morphological and histochemical variations in the different stages of their functional period and in the different seasons.

2 cm square blocks of the bark containing the cambium and the conducting phloem were removed from the main trunks of woody plants, using a chisel and hammer, fixed in F.A.A. for five days, and then thin tangential slices (0.5 mm thickness) were made. The above slices were then treated with 5.0% NaOH solution for 3–5 days at 45–50° C, to soften the tissues. After 72 hrs, they were transferred to fresh NaOH solution of the same concentration. Periodical checking was made to know the condition of the treated slices. The treatment was continued till the cells of the slices became sufficiently loose to allow the separation of the individual elements on a slide when a slight pressure is applied over the coverslip after mounting.

When the desired stage was reached, the slices were washed and stained in 1% aqueous solutions of either astra blue or lacmoid for 12–24 hours, depending on the season of the collection. The latter was preferred when the collection was made in the winter, in order to make clear the closure of the sieve pores by callose. The stained slices were either mounted in glycerine or dehydrated in ethanol in quick succession and mounted in Canada balsam for examination. The technique gave excellent results. Some of the sieve elements obtained by this technique are shown in Figs. 1 and 2. All the morphological details including the protoplasmic contents and the callose deposition in the winter collections (Fig. 1) are clearly seen in the mace-



FIGS. 1–2. Fig. 1. Some macerated sieve tube members of *Cassia javanica* Linn. (winter collection) having terminal sieve plates and lateral sieve areas plugged with callose. CC, Companion cell; CP, Callose plugs, SE, Sieve element,  $\times 130$ . Fig. 2. A single sieve tube member of *Cassia fistula* Linn. with open pores (summer collection). SA, Sieve area, SP, Sieve plate,  $\times 520$ .

rated elements when stained with astra blue or lacmoid.

The technique could be used in the class-rooms to demonstrate the three-dimensional forms of the sieve elements together with their morphological and histochemical characteristics, developing in the various seasons.

The second and third authors [Mohd. Yunus and (Miss) Farzana Farooqui] are very thankful to the Council of Scientific and Industrial Research, New Delhi, for the award of Senior and Junior Fellowships respectively. The laboratory facilities provided by Professor R. Khan, Head of the Botany Department, Aligarh Muslim University, are being gratefully acknowledged by all.

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# STUDIES ON UTILIZATION OF NITROGEN BY FOMES DURISSIMUS LLOYD FROM WOOD OF SWIETENIA MAHOGANI, CASUARINA EQUISETIFOLIA AND MIMUSOPS ELENGI

INFORMATIONS concerning the nitrogenous materials in wood are yet scanty largely because of the fact that very meagre amount of nitrogen is present in wood. This small amount of nitrogen is, however, of paramount importance to wood-destroying micro-organisms that live in wood and derive their nitrogen requirements for nourishment from wood. In general, higher percentage of nitrogen exists in angiosperms than in gymnosperms<sup>1</sup>, in sapwood than in heartwood<sup>2-3</sup> and susceptibility of wood to decay is known to be directly related to such natural variation in the nitrogen content<sup>4-5</sup>. The present investigation has been undertaken with a view to study the utilization of nitrogen by *Fomes durissimus* Lloyd from wood of *Swietenia mahogani*, *Casuarina equisetifolia* and *Mimusops elengi* on the basis of quantitative estimation of total nitrogen contents.

For quantitative estimation of nitrogen, wood blocks were prepared from adjacent end-matched specimen obtained from a particular annual increment of sapwood of each host stem of about 30 cm in diameter. Heartwoods, obtained from region of about 15 cm from the pith were selected similarly from end-matched specimen.

The mycelium of *F. durissimus* was isolated from fructifications growing luxuriantly on standing trees

of *S. mahogani*, *C. equisetifolia* and *M. elengi* in the Burdwan University Campus, Burdwan, West Bengal. Pure culture of the test-fungus was made on malt-agar medium in Kolle flasks and sap and heartwood blocks of all the host-species were exposed to the mycelia of *F. durissimus* for decay under controlled conditions<sup>1</sup>. The test blocks after four and eight months decay were freed from thick superficial mycelial mat. Sound and decayed wood blocks of aforesaid host-species were separately cut into small chips, ground into a fine powder of about 40 mesh and used for quantitative estimation of nitrogen.

For estimation of total nitrogen colorimetric method<sup>7</sup> was employed. Dry wood meal (10 mg) was digested with 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> for 1 hour and then 0.8 to 1 ml of H<sub>2</sub>O<sub>2</sub> was added and the digestion was continued until the solution became completely clear. Its volume was made upto 10 ml and finally diluted ten times to make the volume to 100 ml by addition of distilled water.

5 ml of alkaline Nessler's reagent and 1 ml of 10% NaOH and 10% Na<sub>2</sub>SiO<sub>3</sub> mixture (1:1) were added to 1 ml of the aliquot. Intensity of the colour developed was measured colorimetrically after 10 minutes at 430 mμ and the total nitrogen content was expressed as μg/100 mg dry weight from which percentage was calculated.

The results are given in Table I.

TABLE I  
*Changes in total nitrogen content of wood of  
S. mahogani, C. equisetifolia and M. elengi  
due to decay by Fomes durissimus Lloyd  
after four and eight months*

Nature of wood	*Total nitrogen in sound wood (%)	* Total nitrogen in decayed wood			
		4 months		8 months	
		Per- cent- age	Loss (%)	Per- cent- age	Loss (%)
<i>S. mahogani</i>					
Sapwood ..	0.413	0.205	50.6	0.048	88.3
Heartwood	0.391	0.275	29.6	0.133	66.0
<i>C. equisetifolia</i>					
Sapwood ..	0.213	0.120	43.2	0.093	56.3
Heartwood	0.113	0.073	35.4	0.061	47.7
<i>M. elengi</i>					
Sapwood ..	0.300	0.111	63.0	0.050	83.3
Heartwood	0.216	0.120	44.4	0.078	63.1

\* Average of three replicates.

From Table I, it appears that total nitrogen contents of sound wood of three host-species under consideration differ considerably, being maximum in *S. mahogani* and minimum in *C. equisetifolia*. Moreover, the sapwood of all the hosts shows

much higher nitrogen content than the respective heartwood because of comparatively high level of nitrogenous constituents in the cytoplasm of the living cells present in the sapwood<sup>2-3</sup>. Considerable reduction in the total nitrogen content becomes evident in all the types of wood when exposed to the attack of *F. durissimus* for 4 months. Further reduction in nitrogen content of wood occurs as the period of decay increases from four months to eight months. Of the three host-species, the percentage of loss in nitrogen content is maximum in *S. mahogani* where original nitrogen content is maximum while *C. equisetifolia* having minimum nitrogen content shows minimum loss.

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#### DEVELOPMENT OF TOMATO PLANTS FROM SHOOT TIPS

THE apical meristems of shoots have the ability to regenerate into plants, grown under *in vitro* conditions. Robbins<sup>1</sup> was successful in cultivating the shoot apices of pea, maize, and cotton. LaRue<sup>2</sup> cultivated small fragments, containing cotyledon-base and apical buds of dandelion, chrysanthemum, lettuce, and tomatoes. The buds differentiated into shoot and roots. Apical buds of dodder were grown by Loo, Bertossi, and Baldev<sup>3-5</sup>. Taking advantage of the fact that apical meristems are 'virus-free', Morel and Martin obtained healthy clones from apical meristems of dahlia, and potato<sup>6-7</sup>.

We attempted to grow the shoot tips of *Lycopersicon esculentum* Mill., var. Col under *in vitro* conditions to obtain healthy plantlets/clones. One hundred shoot apices, ca 1 mm long, from 10 day-old tomato seedlings, were planted aseptically into test-tubes (7.5 × 2.5 cm) containing 20 ml White's medium supplemented with coconut milk (15% V/V), and solidified with 0.7% agar. The cultures were grown at 26° ± 1° C and subjected to artificial illumination of 3200 lux for 10 hr/day. The cultures were repeated 5 times,

After a lag phase of 10–15 days, callus formation was observed on the basal end of shoot apices. In 73% cultures, the remaining cultures did not show any growth. Of the former, 20% did not differentiate into organs, while 50% explants developed into leaves, or leaf-like organs, from the callus. There were numerous shoot apices with a number of primordia in various stages of development. However, no root or stem formation was observed. Observations were made by Al-Talib and others in *Pseudotsuga taxifolia*.

Only after 35–40 days of inoculation explants formed roots and shoot. Thereafter a normal stem with 4–6 leaves. The differentiating callus developed into stem followed by root formation only after the differentiation of shoot system. Chalakyan<sup>9</sup>, in his experiments on *Rudbeckia*, observed stem growth and flower initiation after root regeneration. He discusses the role of auxin in the regulation of stem growth, and leaf initiation. Butenko<sup>10</sup>, working with *Perilla*, also observed dependence of stem growth on the root system. Our studies indicate that the establishment of root system is important for the growth and ontogeny of plants grown from isolated shoot apices.

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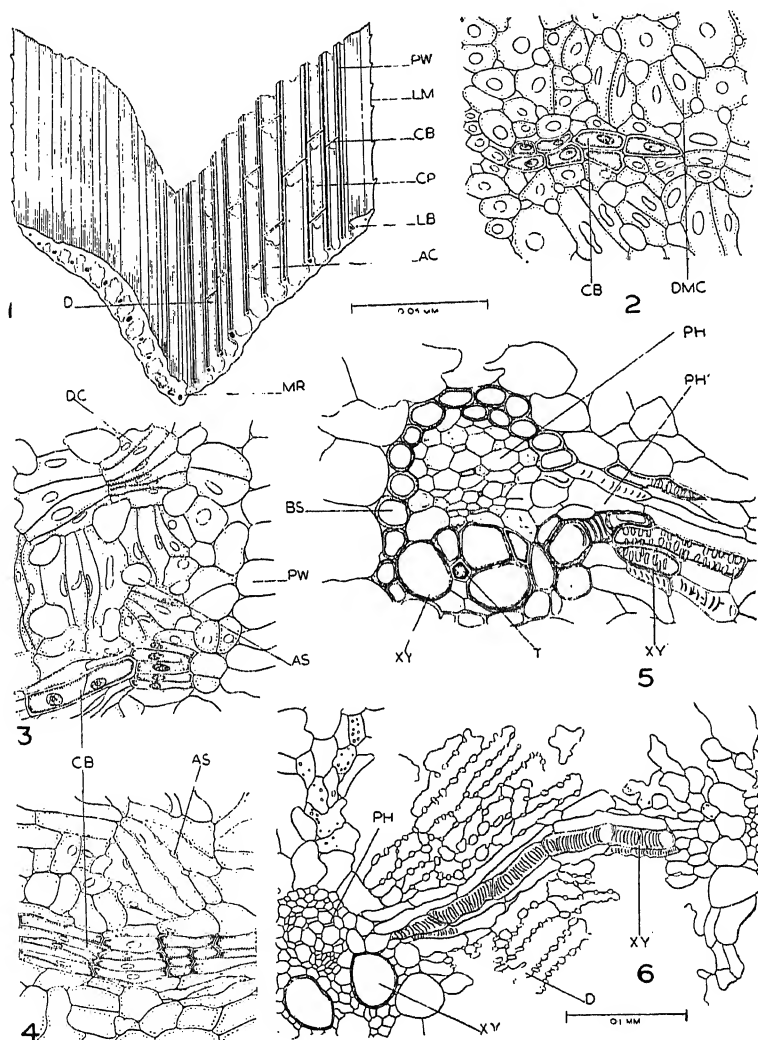
#### DIAPHRAGMS IN CYPERUS

DIAPHRAGMS occur in a few members of the Cyperaceae, like *Equisetum* and ferns (Leitgeb, 1868). They occur frequently in some dicotyledonous families, and numerous monocotyledonous families (Snow, 1914, 1920). In Cyperaceae the diaphragms have been reported in several marshy plants (Sifton, 1945; Shiam, 1963; Mehra and Shah, 1963; Metcalfe, 1971). The air cavities, some Cyperaceae, are traversed by diaphragms.

consisting of translucent cells that fail to breakdown (Metcalf, 1971).

Structurally, the diaphragms are the partitions which break the continuity of air passages (Leitgeb, 1857); Snow (1914) has, however, used the word 'diaphragm' for the perforated structures, one to several layers thick. They cross the air passages at regular intervals.

The development of air cavities, and the diaphragms, takes place simultaneously. In the very young leaves of *Cyperus esculentus* there is no sign of air cavities, and diaphragms. But, later on, as the leaf grows in size, the cells, where these cavities arise, begin to lose their cytoplasmic contents and the nuclei degenerate. Ultimately, their walls breakdown and dissolve forming small air cavities. These



Figs 1-6. Fig. 1. Diagrammatic representation of air canals obliterated by diaphragms. Fig. 2. Diaphragm mother cells, and procambial strand of a cross bundle. Fig. 3. Division of diaphragm mother cells. Fig. 4. Air spaces formed by separation of middle lamella of diaphragm cells. Fig. 5. T.s. of longitudinal bundle and its connection with a cross bundle. Fig. 6. T.s. of leafy bract showing diaphragm and cross bundle.

AC, air canal; AS, air space; BS, bundle sheath; CB, cross bundle; CP, compartment; D, diaphragm; DC, diaphragm cells; DMC, diaphragm mother cell; LB, longitudinal bundle of leaf; LM, leaf margin; MR, midrib; PH, phloem of longitudinal bundle; PH', phloem of cross bundle; PW, partition wall; T, tannin; XY, xylem of the longitudinal bundle; XY', xylem of the cross bundle.



cavities run longitudinally and alternate with the vascular bundles, and remain separated from one another by multicellular partition walls. The cavities are interrupted, at irregular intervals, by transverse diaphragms, one to several cells thick. The cells of diaphragms are polygonal to stellate, and always accompanied and supported by small cross bundles given off from the main longitudinal bundles of the leaf. The cross bundles are very prominent in *C. esculentus*, and composed of xylem and phloem connected with the xylem and phloem of the main longitudinal bundle (Fig. 5).

The development of a diaphragm (Fig. 1) is initiated by divisions of parenchymatous cells interrupting the air canals (Fig. 2). Successive divisions in the same mother cells result in the formation of 4-6 tiers of cells (Fig. 3). The formation of the dividing walls is parallel to the long axis of mother cells. Later, the middle lamellae

begin to separate, at a number of places, from the adjoining cells, resulting in the formation of small perforations (Fig. 4). These perforations enlarge considerably, and give a beaded appearance to the cells of the perforated diaphragm (Fig. 6).

School of Plant Morphology,

R. SHIAM.

Meerut College,

O. P. SHARMA.

Meerut (India), February 11, 1974.

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6. Snow, L. M., *Bol. Gaz.*, 1914, 58, 495.
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## SHORT SCIENTIFIC NOTES

### Occurrence of *Eulecanium* sp. *tiliae* (L.) (Homoptera: Coccidae) on Plum and Apple in Himachal Pradesh

Plum is an important fruit crop in subtemperate regions of India. During the year 1970, large number of plum trees in Solan (1,500 M.asl) area of Himachal Pradesh were found infested with a coccid which was identified as *Eulecanium* sp. *tiliae* by the British Museum of Natural History, London. Besides plum, the insect was also observed on apple at Kalpa in Himachal Pradesh. It is believed to be the first record of *E. sp. tiliae* from India.

The female adult scale is dark brown, hemispherical and devoid of legs and antennae. Ventral surface of the scale remains intact with the host surface till the hatching of eggs. Oviposition is prolonged and eggs are concealed beneath the female scale, where they hatch. After oviposition female scale dies. The scale lays eggs from mid-March to beginning of April and hatching takes 12-15 days. Young crawler is light brown in appearance with long thread-like beak and possesses legs and antennae. Reproduction is parthenogenetic and males were not recorded. Young crawlers migrate to the undersurface of leaves where they suck sap. The crawlers migrate back to twigs during October-November, when trees start shedding off leaves. Life-cycle is completed in one year.

Effective check of the scale is kept by its natural enemy, *Coccophagus* sp. nr. *ishii* (Hymenoptera; Aphelinidae). The parasitism was as high as 73%.

Department of Entomology,

R. C. MISHRA.

H.P. University,

O. P. BHALLA.

College of Agriculture,

Solan, February 12, 1974.

### Observation on the Host Specificity of Isopod Parasite *Nerocila* Sp. from Andhra Coast

During investigations on clupeoid fishes of the Andhra coast, it has been observed that the parasite *Nerocila* sp. is found on *Ilisha melastoma* (Schneider, 1801); the place of infection being the gill chamber. Meenakshisundaram (1945) has recorded the host specificity of this parasite from Kutch on the West coast. While examining the landings he found this parasite on *Ilisha indica* (= *I. melastoma*) but not on *I. filigera*. It is further confirmed that the parasite is specific in the selection of its host, because it has not been found on other species like *I. whiteheadi* from Kakinada, *I. elongata* from Masulipatnam, *I. megaloptera* from Visakhapatnam and Suryalanka and *I. filigera* from Gollapalem and Masulipatnam. In all, 300 specimens of the above species were examined. The parasite has been found on 9 out of 25 specimens of *I. melastoma*.

Department of Zoology,

B. V. SESHAGIRI RAO.

D.N.R. College,

Bhimavaram-534202, April 5, 1974.

1. Meenakshisundaram, P. T., *J. Mar. biol. Ass. India*, 1965, 7 (1), 202.

### Three New Host Records of Leaf Spot Fungi

The authors noticed leaf spot incidence on *Pedaliium murex*, L., *Tridax procumbens*, L. and *Schefflera stellata* Harms. in Tirupati area (A.P.), during September to November months of 1971 and the details are reported briefly herein.

1. *Leaf spot of Pedaliium murex* L.—A mild leaf spot disease was noticed both on young and older leaves. Spots appeared dark brown, depressed 2–3 mm in size and occasionally the spots of concentric zonations was noted. The causal organism was isolated and has been identified as *Corynespora cassicola* (Berk and Curt) Wei, and the culture has been deposited in CMI, Kew, England (IMI 173332).

2. *'Shot-hole' of Tridax procumbens* L.—The spots appeared water soaked initially which enlarge upto 5–6 mm in diameter, with a faint yellow margin. The mature lesions appeared depressed and brittle. The necrotic area may drop off giving a 'Shot-hole' appearance on the leaf. The causal organism has been identified as *Sclerotium rolfsii*, Sacc.

3. *Leaf blotch of Schefflera stellata*, Harms.—Leaf spots appear pale yellow, 1–2 mm in size with a dark margin. More frequently infection was noticed on the margins of the leaflets. Adjacent lesions coalesce resulting in larger depressed necrotic blotch, appearing pale brown with numerous acervuli. Necrotic streaks were noticed on young stems also. Isolations were made from young non-sporulating lesions and based on the morphological characters the fungus has been identified as *Colletotrichum gloeosporioides*, Penzig. and its perithecial stage as *Glomerella cingulata* (Stonem) Spauld and Schrenk. The culture has been deposited in CMI, Kew, England (IMI 173331).

Department of Botany,

P. SUBRAHMANYAM.

S.V. University,

Y. R. SARMA.\*

Tirupati-517502 (A.P.),

February 26, 1974.

\* Present address : Division of Plant Pathology, CPRI, Kasargod, Kerala.

### A New Leaf Spot Disease of Mulberry in India

During the course of investigation on the leaf-pathogenic fungi of Meerut and its neighbourhood, a new leaf spot disease of mulberry (*Morus alba* L.), growing in the Meerut College, Meerut Campus, was observed in the month of November, 1973. Diseased leaves in the beginning showed small, roundish, pale brown spots on their abaxial side.

Such spots at a later stage became almost dark brown in colour, a few of which might coalesce with each other, covering almost whole of the laminar surface. Such leaves turned brown in colour, became dry and soon fell off from tree branches.

The microscopic examination of the transverse sections of leaves through leaf spots and of the preparations from such spots revealed the presence of mycelium, conidiophores and conidia of a fungus, the morphologic characteristics of which are being summarised below.

Mycelium thin-walled, at first hyaline becoming darker later, 2.6–5.2  $\mu$  diam.; conidiophores emerging through stomata or more commonly from between the epidermal cells, singly or mostly in tufts of 3–5, simple, slender, deep-olivaceous, slightly swollen at the apex, with distinct geniculations due to sympodial growth, 3–8 septate, 20.8–78  $\times$  5.2–9.1  $\mu$ ; conidia (porospores) brown to dark brown, cylindrical, fusiform or obclavate with apical and basal cells rounded, marked by distinct scar at the base, straight or slightly curved to one side, 2–8 septate, 18.2–71.5  $\times$  3.9–9.1  $\mu$ , germinating from polar as well as middle cells.

The fungus was repeatedly isolated on P.D.A. and the pathogenicity was successfully tested on healthy host leaves under artificial culture inoculation experiments. On the basis of above morphologic characters, the causal organism was identified as *Drechslera yamadai* (Nisik.) Subram. and Jain. In India<sup>1,2</sup> as well as abroad<sup>3</sup> this fungus (as *Helminthosporium yamadai* Nisik.) is known to occur so far only on monocotyledonous hosts. Therefore, the present report is the first record of this fungus on any dicotyledonous host like mulberry.

The herbarium specimen and material of the fungus have been deposited in the Cryptogamic Herbarium, School of Plant Morphology, Meerut College, Meerut, India (CH, MCM 201).

Thanks are due to Dr. V. Singh, Reader and Head of the Department, for facilities and encouragement.

Department of Botany,

P. D. SHARMA.

Meerut College,

Meerut, India, April 19, 1974.

1. Misra, A. P. and Prakash, O., *Indian J. Mycol. and Pl. Path.*, 1972, 2, 95.
2. — and Mishra, B., Prakash, O., Dutta, K. K. and Singh, R. A., *Indian Phytopath.*, 1972, 25, 428.
3. Nisikado, Y., *Ber. Ohara Inst. Landw. Forsch.*, 1929, 4, 111.

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## REVIEWS AND NOTICES OF BOOKS

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**The Physics of Phonons.** By J. A. Reissland.  
(John Wiley and Sons Ltd., London), 1973.  
Pp. xi + 319. Price £7.00.

The first four chapters deal with the basic concepts of phonons and unlike the many existing textbooks on lattice dynamics, a clear discussion of the topics like polarization of the lattice waves, periodic boundary condition and the thermodynamics of melting, is given in this book. However, in a book specially devoted to phonons and published in 1973, one expects a more detailed account of the topics like the shell model, the breathing shell model and the calculation of the phonon distribution curves with special reference to Van Hove singularities. These are the basic concepts to the entire field of lattice dynamics and unfortunately are not treated in detail. Appendix G on shell model explains the calculations using this formalism and does not throw any light on the physics of model. Even the examples of frequency distributions are not representative of the modern calculations which reveal the full structure of the  $g(\omega)$  curves.

Chapters 5 to 8 are welcome deviations from the standard texts. These are very useful for graduate students planning research in anharmonic properties. The diagram techniques and the Green's function formulation are elaborately described. This is followed by a good summary on the lattice dynamical treatment of interesting phenomena.

In the chapter on the interaction of phonons with other "ons", the discussions on inelastic neutron scattering are very brief. Perhaps a few more pages with explanations should have been included about this very important phenomenon. No comparison, with experimental data regarding optical or neutron experiments, is given and certain statements (as in page 245, last paragraph) are used in very general terms.

On account of the rather high cost of this book, one has to depend on the library copies for a general study or reference. N. KRISHNAMURTHY.

## ANNOUNCEMENTS

### Xth International Shock Tube Symposium

The Tenth International Shock Tube Symposium will be held in Kyoto, Japan, during 14-17 July 1975. Papers on the application of shock tube to current problems; shock structure and wave interactions, shock in liquids, solids and stratified media; implosions and explosions; laser and its kinetics; high speed flow and boundary layer phenomena in shock tubes, etc., are welcome. Titles must be submitted by 1st October 1974, preliminary manuscripts by 15th January 1975 and complete manuscripts by 16th July 1975. Address for correspondence: Tenth International Shock Tube Symposium, Kyoto University, Kyoto, Japan.

### Award of Research Degree

Karnatak University, Dharwar, has awarded the Ph.D. degree in Zoology to Shri S. N. Hegde for his thesis entitled "Studies on the Domestic Fowl— with reference to the Role of the Caeca Gamma-Ray Irradiation of developing chicks and chick nutrition".

---

### Books Received

*Immunology Series* (Vol. 1)—*Mechanisms in Allergy*. Edited by L. Goodfriend, A. H. Schon and R. P. Orange. (Marcel Dekker, Inc., 95, Madison Avenue, New York, N.Y. 10016), 1973. Pp. xviii + 578. Price \$26.50.

*Fertilizer Science and Technology Series* (Vol. 2)—*Ammonia* (in 4 parts). Part I. Edited by A. V. Slack and G. Russell James. (Marcel Dekker, Inc., 95, Madison Avenue, New York, N.Y. 10016). Pp. xiii + 414. Price \$39.50.

*Solid-State Circuits*. By G. J. Pridham. (Pergamon Press, Ltd., Headington Hill Hall, Oxford), 1973. Pp. x + 184. Price not given.

*Basic Electrotechnology*. By H. Cotton (The Macmillan Press Ltd., London), 1973. Pp. vii + 312. Price £2.50.

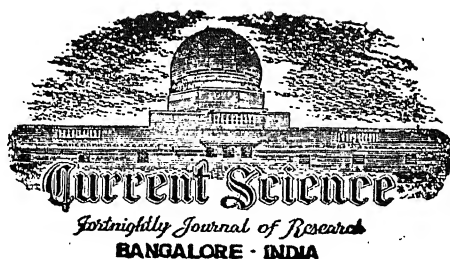
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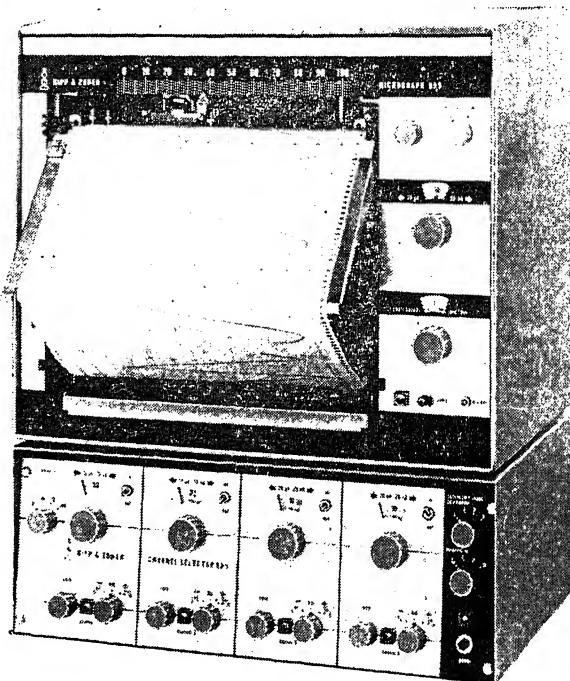
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# GEOPHYSICAL INVESTIGATIONS FOR DEVELOPMENT OF GROUNDWATER IN PARTS OF GUNTUR DISTRICT, ANDHRA PRADESH

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*G.S.I., Central Region, Nagpur*

AND

T. S. RAMAKRISHNA

*G.S.I., Western Region, Jaipur*

## ABSTRACT

Valuable information on the sub-surface geological feature was brought out in parts of Guntur District while carrying out geophysical investigations for groundwater employing seismic refraction and electrical resistivity methods during the Field Season 1967-1969. The electrical surveys were chiefly useful in demarcating more favourable zones for groundwater exploration. In the portions of thick clay cover, the seismic refraction surveys were able to delineate the Chebrole-Tangellamudi sandstone in-lie over an area of 1,200 sq. km. at depths varying from 20 to 100 metres.

A crystalline ridge which is partly controlling the groundwater flow was indicated in the middle of the basin extending over a distance of 30 km between Ponnur and Chivalur. In the present paper, the results of the surveys are discussed in the light of Oil and Natural Gas Commission (O.N.G.C.) and Exploratory Tubewell Organisation (E.T.O.) borehole data.

## INTRODUCTION

THE Andhra Pradesh State Government had launched a plan to sink tubewells in the Coastal Districts of Guntur, Krishna, East and West Godavari Districts. Geophysical investigations for groundwater in parts of Guntur District were undertaken by the senior author for recommending suitable sites for tubewells (Fig. 1). The investigation was aimed at studying the sub-surface features such as the sandstones outcropping at Chebrole-Tangellamudi and to determine the thickness of sediments.

In all about 100 seismic profiles (shot-detector spread 1.2 km) and 200 electrical probes employing Wenner Configuration (maximum electrode separation 200 m) were conducted.

## GEOLOGY

The area represents a basin possibly filled with sediments derived from the crystallines exposed west of Guntur and all along National Highway-5. The crystallines comprise of Charnockites and Khondalites. Isolated patches of sandstone (near Chebrole) and conglomerate (near Ippattam) are seen outcropping, 12 km east of the crystallines. Except these, the rest of the area is either covered by black cotton soil or alluvial deposits.

The sandstones of this area devoid of fossils are generally friable and exhibit various colours, viz., pink, red and buff. The sandstones are found to have a gentle dip towards southeast and belong to Gondwana age. According to Jacob the outcrops at Ippattam and Chebrole are lithologically same and the possibility of these beds belonging to "Tertiary" is not unlikely.

## RESULTS AND DISCUSSIONS

The seismic surveys have invariably picked up the discontinuity between the unconsolidated sediments (wet zone of clay, sand, etc., 1200 to 1800 m/sec.) and the consolidated sediments in the present case sandstones, over the entire area (2000 to 2500 m/sec.). At some places velocities ranging from 3000 to 3500 m/sec. were also encountered possibly corresponding to shales or limestones (Fig. 2). It is further observed that it would not be possible to identify the sandstone belonging to different ages and they have been picked up as one layer. Comparatively lower longitudinal velocities were recorded for the sandstones in the eastern portions of the area due to the difference in compactness and composition. Crystalline basement was indicated with a longitudinal velocity of 5500 to 6000 m/sec between Ponnur and Chivalur.

Along the section AA (Fig. 3) three layers have been picked up. The top layer with a velocity of 380 to 500 m/sec. corresponds to the soil which varies in thickness from 2 to 10 m. The second layer with a velocity of 1200 to 1700 m/sec. represents the 'Wet Zone' consisting of unconsolidated clays, sands and a mixture of both and its thickness varies from 15 to 60 m. It may be worthwhile to mention that the same order of velocity is recorded for the weathered sandstone around Chebrole. The third layer with a longitudinal velocity of 2000 to 2500 m/sec. corresponds to sandstone and traced over the entire section of 22 km. An intermediate velocity of 3500 m/sec. was encountered near Budampadu at a depth of 160 m which possibly corresponds to shaly sandstones or

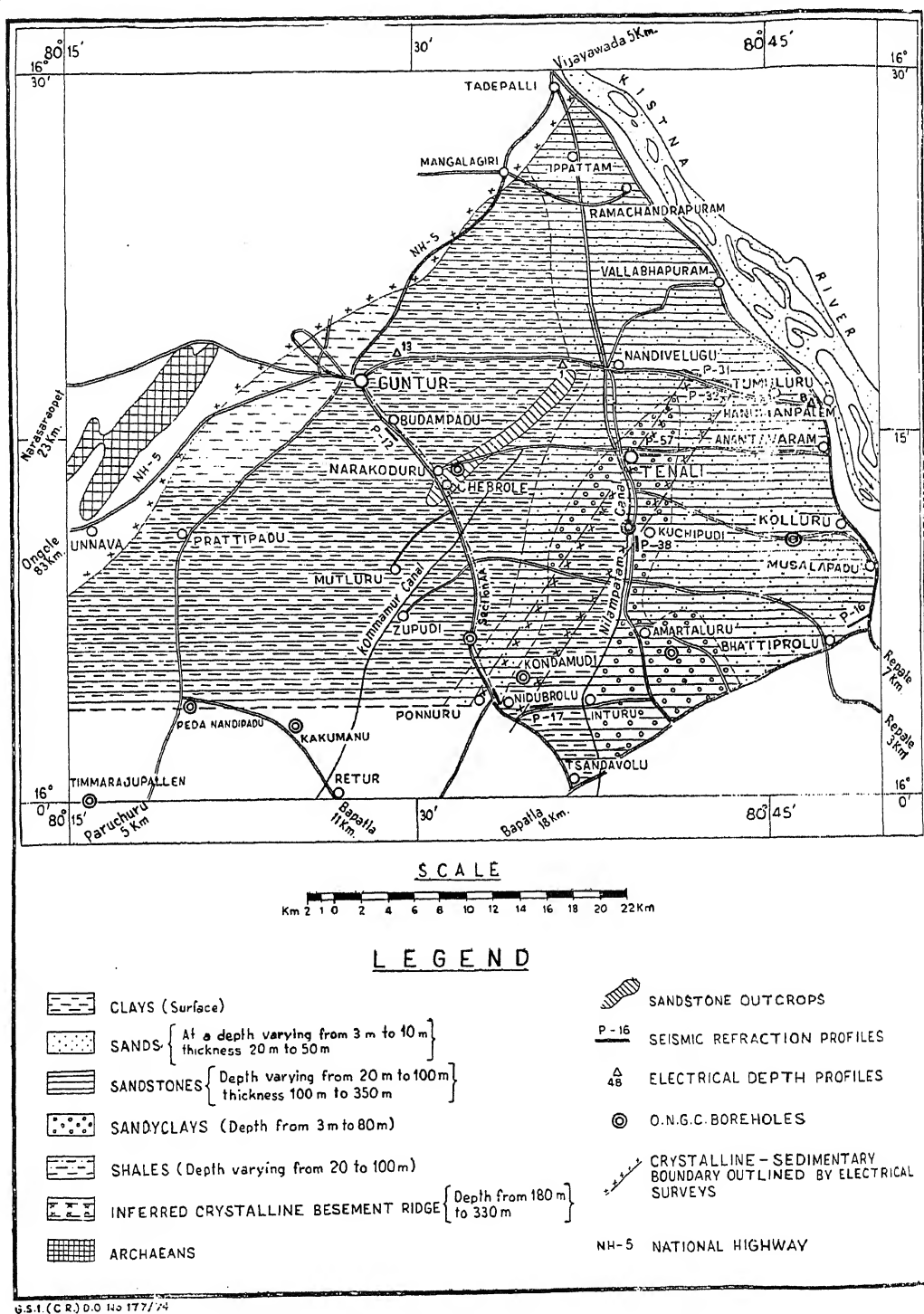


FIG. 1. Location map showing the area covered and the sub-surface geological information inferred from the geophysical surveys in parts of Guntur District, A.P.

limestones. The deposition of sandstone suggests an anticlinal flexure with its axis at Manchala. The O. N. G. C. shallow wells near Muniipalle and Muchipudi have indicated the Mio-pliocene contact at a depth of 42 and 69 m respectively, and this is in agreement with the depths obtained for the top of the sandstone from the seismic refraction surveys. The sandstones outcropping at Chebrole-Tangellamudi area can thus be classified under 'Tertiary'; hitherto they have been believed to be of Gondwana age.

PROFILE 12

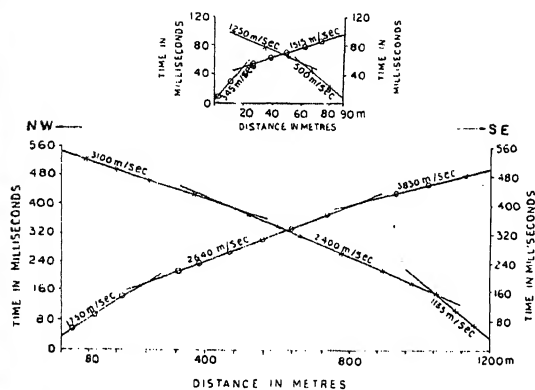
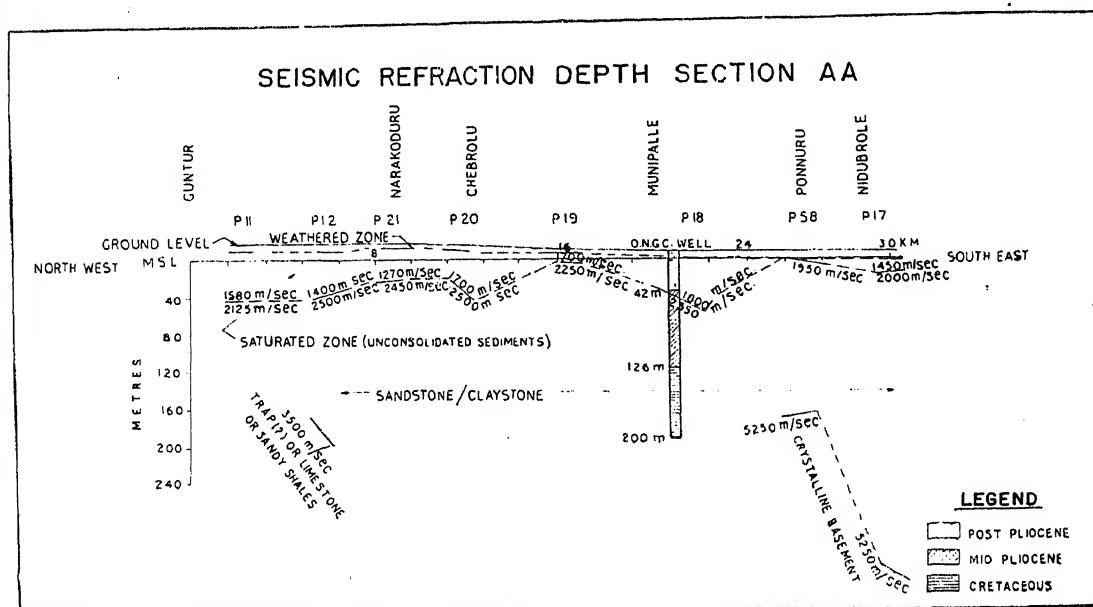


FIG. 2. Typical Time-distance curve in parts of Guntur District, A.P.

One kilometre west of Ponnur, a fourth layer with a velocity of 5250 m/sec. was indicated at a depth of 170 m. This high velocity layer represents the crystallines, occurring at relatively shallow depths in the middle of the basin. Crystalline basement was also indicated over profile P-30 to P-32, P-57 (Chadallavada), P-38 (Kuchipudi), P-51 (Poonnore) and P-17 (Nidubrolu), with depths varying from 175 to 385 m. The crystalline basement indicated in all these profiles align in a N 30° E and the absence of the high velocity layer in the near surroundings suggests a possible crystalline upliftment (Fig. 1). Sandstones (2000 to 2400 m/sec.) layer is found to be extending upto the crystalline basement.

The electrical surveys have been particularly useful in demarcating different alluvial formations, viz., sands (10 to 25 ohm-metres), clays (2 to 4 ohm-metres) and clayey sand (7 to 13 ohm-metres). The depths to various interfaces are arrived from the partial matching of field curves with two layer master curves.

The probe 1 (Fig. 4) was taken over the known exposures of sandstones. This curve represents 'K' type and brought out a 55 m thick sandstone layer (50 ohm-metres) underlain by a conductive horizon (15 ohm-metres). This curve has clearly indicated the presence of claystones within the sandstones. Sandstone (2300 m/sec.) was indicated at a depth of 36 m by refraction seismic surveys at this place.



G.S.I./C.R./D.O No 179/74

FIG. 3. Refraction seismic depth section along AA (Guntur-Nidubrolu road), Guntur District, A.P.



No high velocity layer was indicated with the short-detectors spread of 1.2 km, suggesting considerable thickness of sandstone. Drilling confirmed the presence of sandstone with clay bands upto a depth of 241 m (E.T.O. borehole Narakodur  $16^{\circ} 14' 30'' : 80^{\circ} 03' 00''$ ). The well had to be abandoned due to insufficient thickness of granular zones.

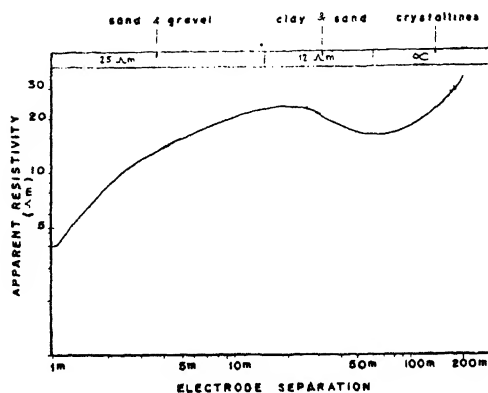


FIG. 4. Electrical depth Probe-1.

The probe 13 (Fig. 5) represents 'H' type and typical of a thick clay layer overlain by thin soil cover. The slight increase in the resistivity values at a depth of 45 m may possibly correlate to the sandstone formation, indicated at a depth of 50 m by the refraction seismic surveys.

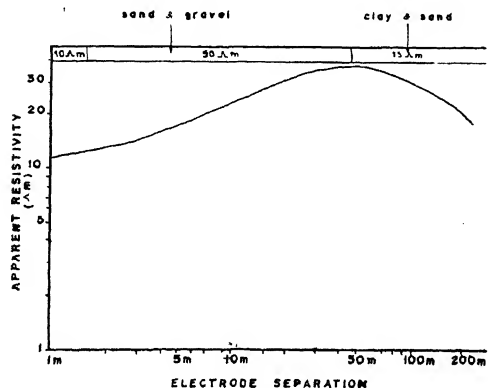


FIG. 5. Electrical depth Probe-13.

The probe 8 (Fig. 6) is selected from a prominent aquiferous zone. A two layer curve was indicated with a good thickness of resistive layer (gravel and sand—25 ohm-metres) overlain by soil cover. It is interesting to note that similar type curves were obtained between Vallabhapuram and Donepudi on the western side of river Krishna. The seismic surveys at this place have brought out

a discontinuity at 30 m (1700/2000 m/sec.) which may possibly correspond to the contact between the unconsolidated/consolidated sediments. The longitudinal velocity of 2000 m/sec. is conspicuously low for sandstones obtained elsewhere in the area. On the other hand it may be mentioned that the electrical method could not distinguish between the sand and sandstones which have been picked up as one layer (25 ohm-metres) in the sounding curves. In view of the persistence of this thick resistive layer over a considerable area (Fig. 1), this zone is recommended for groundwater exploration. Later on, a test borehole put down by the E.T.O. to a depth of 306 m near Musalapadu ( $16^{\circ} 11' : 80^{\circ} 46'$ ) located in this zone proved to be a productive well.

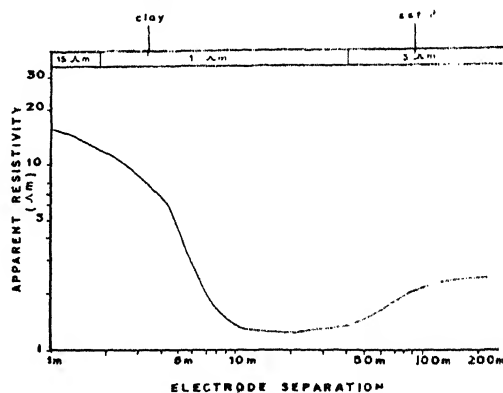


FIG. 6. Electrical depth Probe-8.

The probe 48 (Fig. 7) represents 'KH' type and has brought out two resistive layers. The first layer is attributed to sand/sandstone while the second represents crystalline.

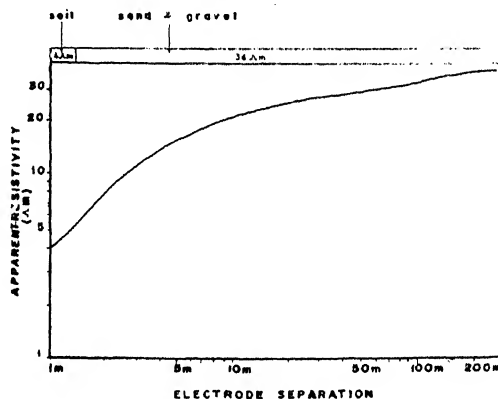


FIG. 7. Electrical depth Probe-48.

#### CONCLUSIONS

The sub-surface geological features inferred both from the seismic refraction and electrical resistivity

surveys are presented in Fig. 1. The surveys have indicated large thickness of sediments. Useful information on the sub-surface disposition of sand, clays, clayey sands, sandy shales, sandstones and crystallines has been clearly brought out. Three pockets of thick clay beds have been outlined around Budampadu, Panchallavaram and Penamarru by electrical surveys. E.T.O. well near Panchallavaram ( $16^{\circ} 15' : 80^{\circ} 59' 30''$ ) has passed through large thickness of clays and yielded poor quality of water.

The seismic surveys have delineated sandstones (outcropping at Chebrole) with intercalations of clays over the entire area to depths varying from 20 to 100 ms. The thickness of sandstones varies from 100 to 340 m. It is further observed that sandstone peters out further south of Varagani. A crystalline basement ridge has been outlined in the NE-SW direction over a distance of 30 km between Ponnuru and Chivaluru. It is not unlikely that this ridge in the middle of the basin occurring at depths varying from 170 to 385 m controls groundwater flow in the sandstones. The E.T.O. well near Kondamudi ( $16^{\circ} 58' : 80^{\circ} 36'$ ) reaching depth of

305 m has intersected mostly clay bands with a few sandy layers, and has not touched the crystalline. This location of the well falls within the boundary of the inferred ridge. If the well had gone to a depth of 350 m or so, possibly the crystallines would have been struck. This well had to be abandoned due to the poor quality of water.

Recent drilling carried out by the Agro-Industries Corporation, Andhra Pradesh in the Varagani, has yielded encouraging results and confirmed the presence of sandstone layer at stipulated depths.

#### ACKNOWLEDGEMENT

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### UPTAKE OF RADIOACTIVITY BY BODY FLUIDS AND TISSUES IN RHESUS MONKEYS AFTER INTRAVENOUS INJECTION OR INTRANASAL SPRAY OF TRITIUM-LABELLED OESTRADIOL AND PROGESTERONE

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**T**HE recurrence of menstrual cycles and ovulation in non-human primates is a direct result of the neurally mediated interaction between the endocrine secretions of the adenohypophysis and the gonads<sup>1,2</sup>. Studies carried out recently have led to the concept that, in addition to the well-documented evidence implicating the involvement of neurons and blood, specialised ependymal derivatives and the cerebrospinal fluid (csf) constitute important cellular and humoral pathways over which the neuro-endocrine regulation of the menstrual cycle is effected<sup>1,3,4</sup>. The finding of sex steroids being transferred into the csf when administered intramuscularly<sup>5</sup> or intravenously<sup>6</sup> and the finding of oestrogen being able to influence gonadotropin secretion when injected into the cerebral ventricles<sup>7</sup> have lent additional support to this concept.

The present studies were carried out to determine whether tritium-labelled oestradiol and progesterone are transferred into the body fluids and

taken up by various tissues when they are administered by intranasal spraying. A comparison of the relative uptake of the radioactivity by different tissues is made between monkeys given these steroids by intravenous injection and intranasal spray.

#### MATERIALS AND METHODS

Eight healthy, intact, adult female monkeys (4.5 to 6.5 kg body weight) in unknown stages of menstrual cycles were used. Four groups of two animals each were either sprayed intranasally (through the right nostril using an atomiser connected to a respiratory pump) or injected intravenously (through the right saphenous vein) with 0.1 mCi of either <sup>3</sup>H-oestradiol-17B (0.32 µg; Specific Activity: 85 Ci/mM) or <sup>3</sup>H-progesterone (0.37 µg; Specific Activity: 84 Ci/mM) dissolved in 0.2 ml of propanediol after anaesthetising the monkeys with sodium pentobarbitone (30 mg/kg body weight). The duration of injection or the

spray was 1 min. The labelled hormones, procured from the Radiochemical Centre, Amersham, U.K., were tested for purity before use. Samples of blood (1.0 ml per sample) and csf (0.25 ml per sample) were drawn before and at various intervals (Figs. 1, 2) after administering the hormones.

tion pattern is that both the steroids are able to enter the csf by 1 min after the administration either by intranasal spray or intravenous injection. While considerable amounts of radioactivity could be detected in the plasma of the  $^3\text{H}$ -oestradiol-sprayed monkeys, the amount of radioactivity in

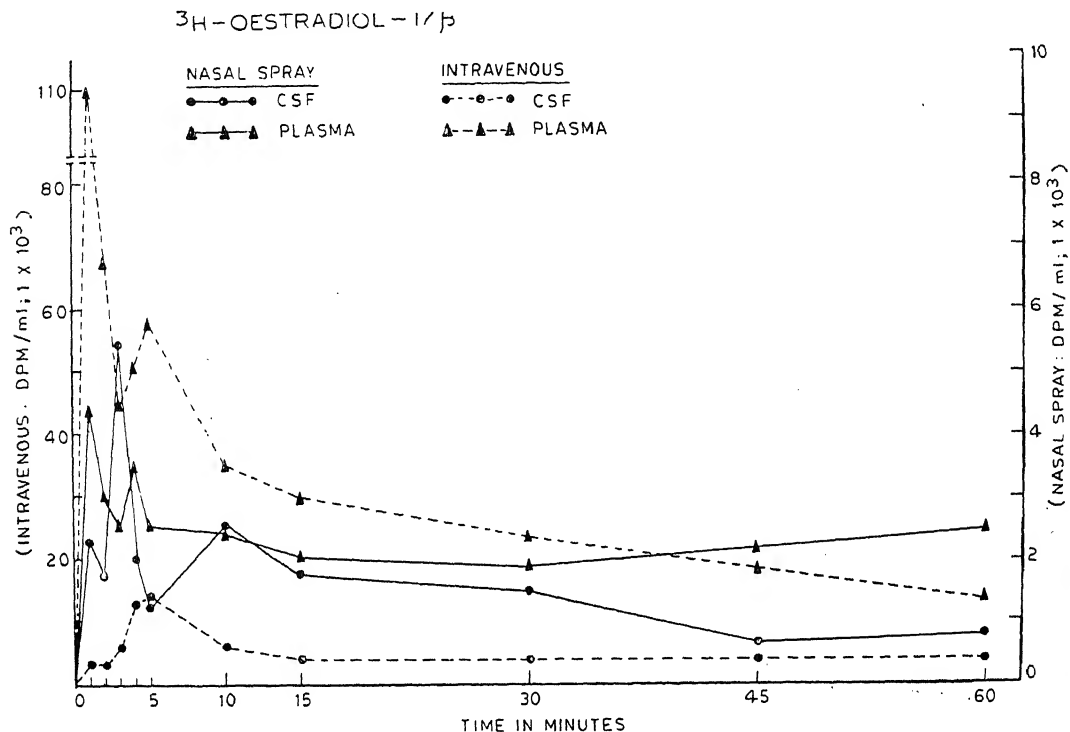


FIG. 1. Temporal distribution of radioactivity in csf and plasma of rhesus monkeys administered  $^3\text{H}$ -oestradiol either intravenously or by intranasal spraying. The radioactivity shown is the average of that obtained for 2 animals.

The animals were killed 1 hr after administering the hormones and tissues listed in Figs. 3 and 4 were taken. The body fluids were processed for estimating the radioactivity in accordance with previously described technique<sup>5</sup>. The tissues were weighed and dissolved in 1.0 to 2.0 ml of 'Soluene-100' (Packard). Radioactivity was estimated in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3320) after adding 10.0 ml of scintillation fluid (PPO and POPOP dissolved in toluene). Radioactivity is expressed as DPM/ml body fluid or DPM/mg tissue by using external standardisation method.

#### RESULTS

The temporal distribution of the levels of radioactivity in the body fluids after administering the hormones by the two routes is shown in Figs. 1 and 2. The interesting feature of the distribu-

tion pattern is that both the steroids are able to enter the csf by 1 min after the administration either by intranasal spray or intravenous injection. While considerable amounts of radioactivity could be detected in the plasma of the  $^3\text{H}$ -oestradiol-sprayed monkeys, the amount of radioactivity in the plasma of the  $^3\text{H}$ -progesterone-sprayed monkeys is negligible. The ratios between the plasma : csf and csf : plasma (Table I) clearly show that the amount of radioactivity is much higher in the csf in the sprayed monkeys as compared with that found in the injected ones. Indeed, the plasma : csf ratio found in the  $^3\text{H}$ -progesterone injected monkeys is reversed in those sprayed with  $^3\text{H}$ -progesterone.

Tissues of all the monkeys showed varying amounts of radioactivity (Figs. 3, 4). The salient difference, however, between the two routes of administration is that in the sprayed monkeys the peripheral target tissues such as the liver, ovary, uterus, vagina and the fallopian tube show much lower amounts of radioactivity in comparison with those injected with the labelled hormones. While the olfactory bulb, olfactory mucosa and respiratory

TABLE I

*Ratio of radioactivity (DPM/ml) in body fluids of rhesus monkeys 1 hr after administering 0.1 mCi of  $^3\text{H}$ -oestradiol-17 B or  $^3\text{H}$ -progesterone*

Hormone	Route Administered	$^3\text{H}$ -oestradiol-17 B				$^3\text{H}$ -progesterone			
		Intravenous		Nasal Spray		Intravenous		Nasal Spray	
Monkey Nos.		455	468	452	470	453	466	459	469
Plasma : csf	..	6.12	8.17	1.22	1.38	15.64	9.25	0.27	0.15
csf : plasma	..	0.16	0.12	0.81	0.71	0.06	0.11	3.70	6.53

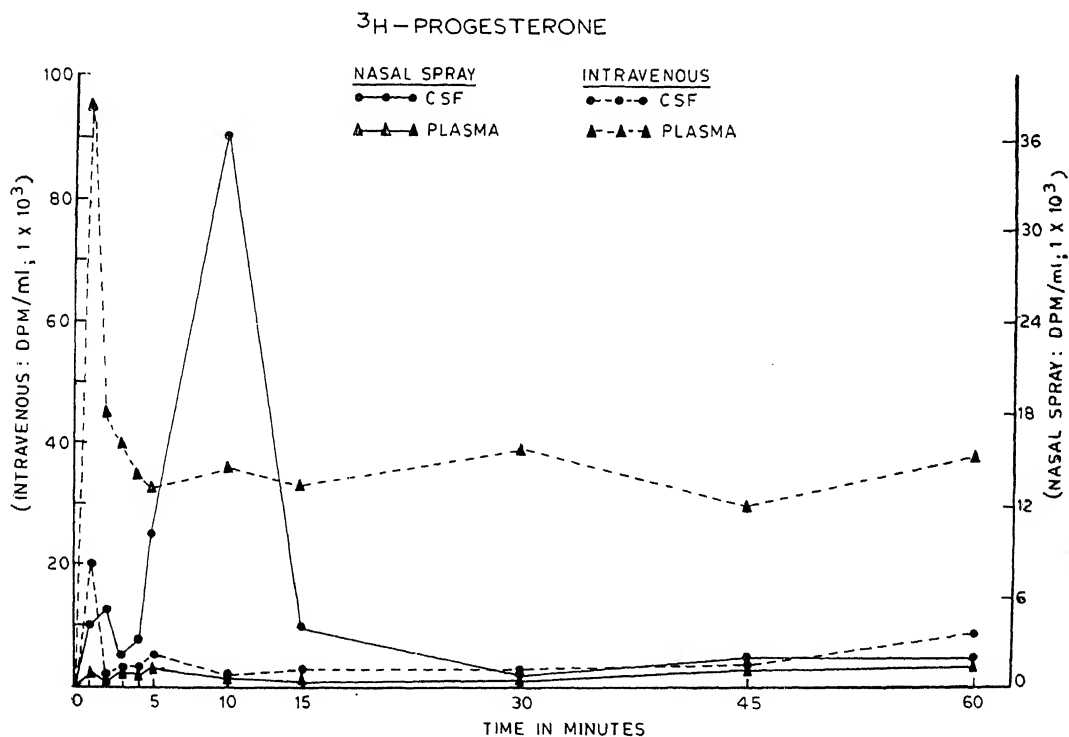


FIG. 2. Temporal distribution of radioactivity in csf and plasma of rhesus monkeys administered  $^3\text{H}$ -progesterone either intravenously or by intranasal spraying. The radioactivity shown is the average of that obtained for 2 animals.

mucosa showed much higher activity in intranasally sprayed monkeys, the amount of radioactivity in the lungs of these animals was surprisingly low as compared with that found in the lungs of the injected animals.

#### DISCUSSION

The results of the present studies must be viewed against the low dose of steroids administered to monkeys whose phases of the menstrual cycle

were not determined. Since the amounts of exogenous hormones taken up by various tissues depend upon the circulating levels of endogenous hormones and since these levels vary in relation to different phases of the menstrual cycle<sup>2</sup>, the amount of radioactivity concentrated by various tissues which reflects hormone-uptake, would vary between different animals. The use of ovariectomised animals and the administration of a standard dose based on the body weight of the animal could

perhaps have considerably reduced this variability between animals. The use of intact animals and low dose of hormones has its own merits in that

liver, ovary, uterus, vagina and the fallopian tube in the sprayed animals is much less in comparison with the injected animals.

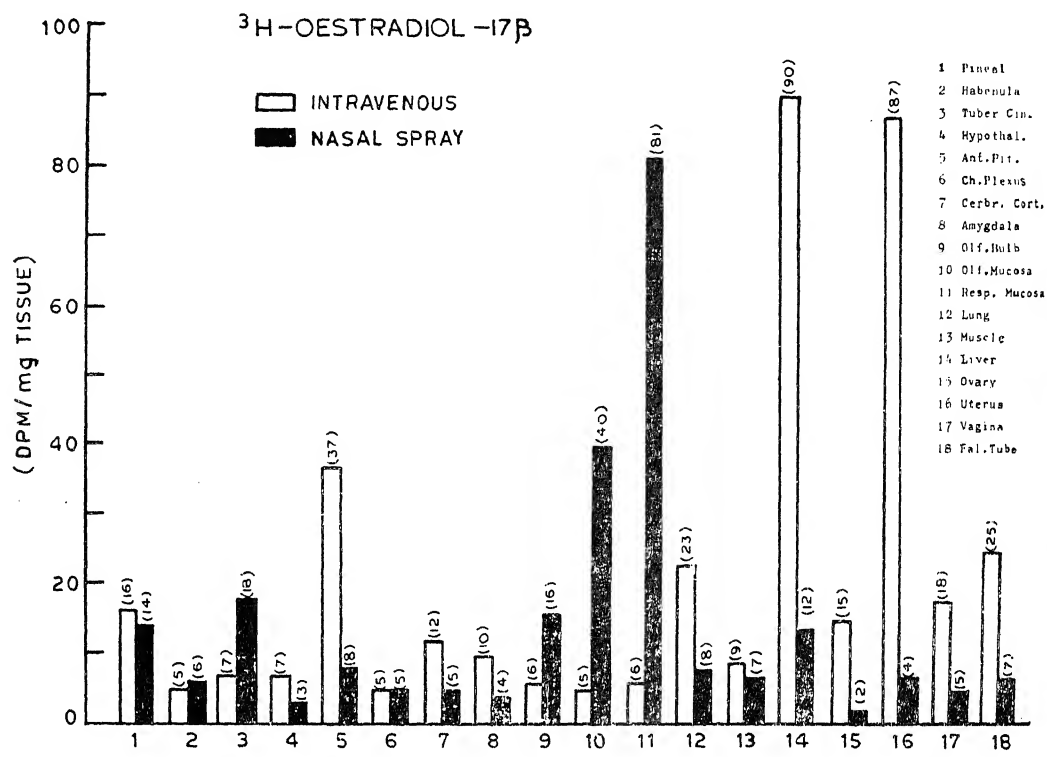


FIG. 3. Radioactivity in various tissues 1 hr after administering <sup>3</sup>H-oestradiol to rhesus monkeys either intravenously or by intranasal spraying. The number in parentheses above each bar represents actual DPM/mg tissue. The DPM is the mean of data obtained for 2 monkeys.

it has provided the kind of answer to the question set out in designing the present investigation, i.e., can exogenous gonadal steroids reach the csf and also other known target organs if they are sprayed intranasally. The use of intact animals would make the results relevant to naturally occurring conditions rather than to their being relevant to 'hormone-starved' condition obtained in gonadectomised animals.

The data obtained from the present investigation clearly indicate that gonadal steroids when sprayed intranasally can enter the csf (and plasma) as quickly as they do when injected intravenously. The amount of radioactivity concentrated by the various tissues examined in the brain is comparable between the two routes of administration except for the anterior pituitary in the <sup>3</sup>H-oestradiol injected monkeys where the amount of radioactivity was much higher in comparison with <sup>3</sup>H-oestradiol sprayed animals. The amount of radioactivity taken up by the peripheral target organs, viz., the

These data support a general inference that the steroids sprayed intranasally preferentially reach tissues known to contain neural mechanisms regulating gonadotropin secretion and they point out a new direction for the future development of fertility regulation technique involving the use of intranasal spray containing naturally occurring steroidal hormones. In this context it would be pertinent to mention that low doses of oestradiol when administered in a manner mimicking the spontaneously occurring increase in endogenous oestrogen prior to the ovulatory LH surge<sup>2</sup> to monkeys as early as day 3 of the menstrual cycle can cause LH surge 12 hrs later<sup>8</sup>. Administration of progesterone to monkeys and women can prevent the spontaneously occurring LH surge and thus inhibit ovulation<sup>9,10</sup>.

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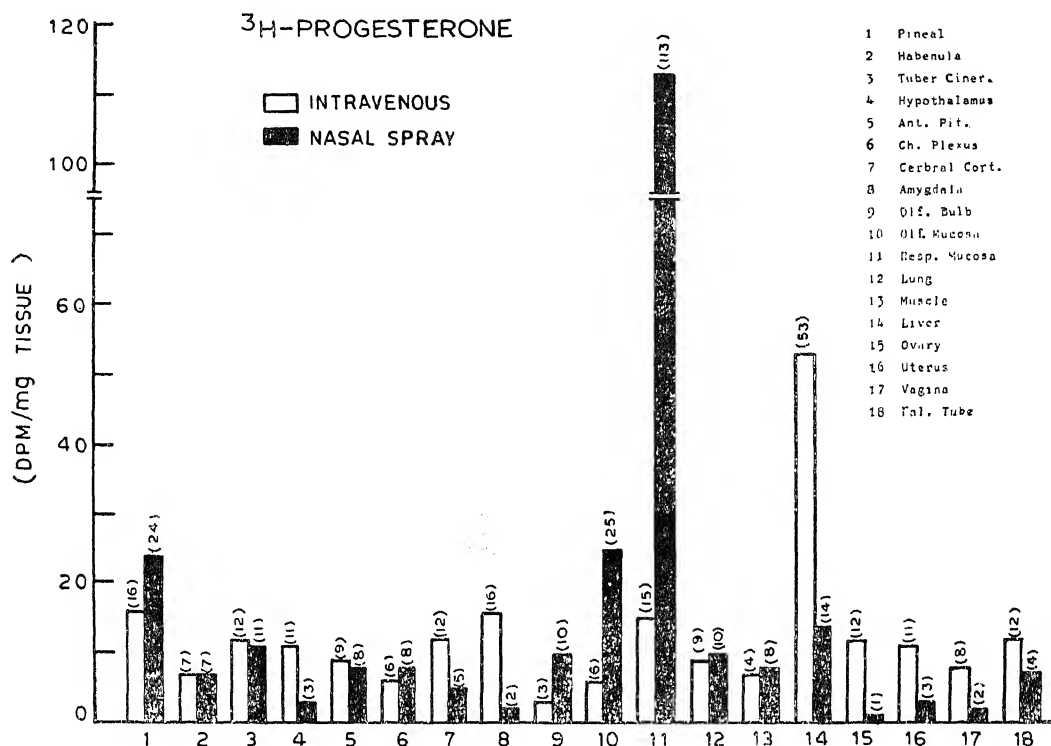


FIG. 4. Radioactivity in various tissues 1 hr after administering <sup>3</sup>H-progesterone to rhesus monkeys either intravenously or by intranasal spraying. The number in parenthesis above each bar represents actual DPM/mg tissue. The DPM is the mean of data obtained for 2 monkeys.

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## LETTERS TO THE EDITOR

NMR STUDY OF INTRA-MOLECULAR MOTION  
IN SOLID 2,5-DICHLOROANILINE

THE crystal of 2-5 dichloroaniline is very useful for the study of the intramolecular mobility of the  $\text{NH}_2$  group as there is no evidence of a hydrogen bond which may hinder such motion. The present study is directed towards examining the feasibility of the free rotation of the amino-group.

## Crystal Data

The crystals of 2,5 dichloroaniline are monoclinic, with space group  $P_{21/c}$ . The cell dimensions are  $a = 13.237 \pm 0.007$ ,  $b = 3.892 \pm 0.006$ ,  $c = 18.803 \pm 0.02$  Å,  $\beta = 135^\circ 13' \pm 11'$ ,  $V = 682 \pm 3$  Å<sup>3</sup> with four molecules in the unit cell. The crystal structure<sup>1</sup> has been determined with the combined application of nuclear quadrupole resonance and X-ray diffraction. Excluding the  $\text{NH}_2$  group, the molecule has a Pseudo centre of symmetry. Since there is no evidence of a N-H...Cl hydrogen bond there is a possibility of the rotation of the amino-group about the C-N bond connecting it to the phenyl ring.

## Experimental Details

The NMR spectrogram of the sample was recorded at Tata Institute of Fundamental Research (Bombay) with the help of 12" magnet, Varian associates variable frequency spectrometer and variable temperature probe assembly. Temperature variations were obtained by suitably regulating the flow of cooled or heated nitrogen gas over the sample. The resonance frequency was 7.5 MC/S. Our sample of 2-5 dichloroaniline was obtained by courtesy of the Central Drug Research Institute, Lucknow, and was highly pure.

## Second Moment Calculations

*Theoretical.*—The rigid lattice second moment consists of two parts (i) intra-molecular contribution  $S_1$  and (ii) intermolecular contribution  $S_2$ . The former is due to the interaction between the protons of the same molecule and may be evaluated by Van Vleck's<sup>2</sup> formula for powder samples

$$S_1 = \frac{716 \cdot 15}{N} \sum_{j > k} r_{jk}^{-6} \quad (1)$$

where  $N$  is the number of protons in the molecule and  $r_{jk}$  is the internuclear distance between the  $j^{\text{th}}$  and the  $k^{\text{th}}$  nuclei. The calculation of  $S_1$ , therefore, involves the construction of a molecular model. In our model of the molecule (Fig. 1)

we have assumed a planar hexagonal benzene ring with C-C, C-H and C-Cl bonds of 1.38, 1.08<sup>3</sup> and 1.70 Å respectively. The N-H bond length and  $\angle \text{HNH}$  were taken as 1.015 Å and  $106.6^\circ$  respectively. This molecular model yielded the value of  $S_1$  as 10.83 gauss<sup>2</sup>.

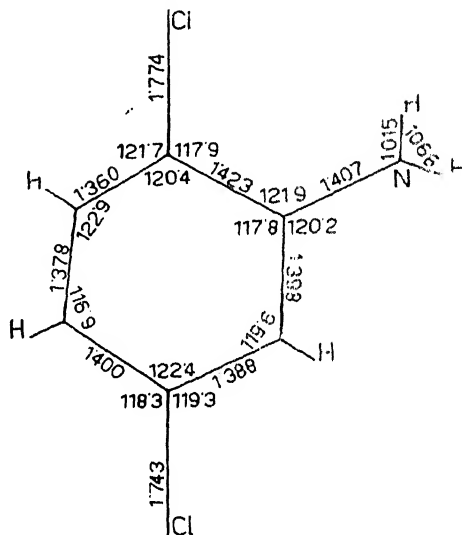


Fig. 1. Molecular structure of 2,5-dichloroaniline

The inter-molecular contribution  $S_2$ , arises out of the interaction between the protons of the different molecules situated at the various lattice sites. The calculation of this part is very tedious but a rough estimate<sup>4</sup> may be made from the expression

$$S_2 = 358 \cdot 1 \times 4\pi N_p (3R^3 V)^{-1} \quad (2)$$

where  $N_p$  is the number of protons in the unit cell,  $R$  is the molecular radius and  $V$  is the lattice volume. Substitution of  $R = 3.56$ ,  $N_p = 20$  and  $V = 682$  Å<sup>3</sup> yields  $S_2 = 12.70$  gauss<sup>2</sup>.

The value of the rigid lattice second moment is, therefore,  $10.83 + 12.70 = 23.53$  gauss<sup>2</sup>.

*Experimental.*—The experimental value of the second moment may be calculated from the trace with the help of the expression

$$S = \frac{1}{2} \left[ \int_0^\infty g'(H) (H - H_0)^3 dH / \int_0^\infty g'(H) (H - H_0) dH \right] \quad (3)$$

where  $H_0$  is the resonance field value. This expression may be simplified to the form

$$S = \frac{\sum h^3 f(h)}{3 \sum hf(h)} \quad (4)$$

by applying the trapezium rule to the integrals. A graph is plotted between the experimental values of the second moment obtained at different temperatures.

#### Discussion

The fairly good agreement between the theoretical value ( $23.53 \text{ gauss}^2$ ) and the experimental ( $21.91 \text{ gauss}^2$ ) as values at  $77^\circ \text{K}$  indicates that the lattice is effectively rigid at the liquid nitrogen temperature. From Fig. 2 we observe that between  $77^\circ \text{K}$

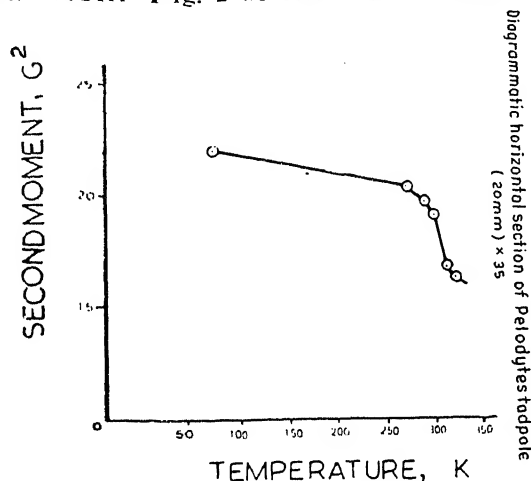


Fig. 2. Second moment vs. temperature curve.

and  $294^\circ \text{K}$  the second moment vs. temperature curve possesses a slight downward slope. This slope may be assigned to the torsional oscillations of the  $\text{NH}_2$  group which are capable of occurring at such low temperatures. Beyond  $294^\circ \text{K}$  there is a rapid fall in the value of the second moment indicating the onset of molecular motion in the crystal lattice.

The molecular motions can take place only after the molecule has been imparted sufficient energy (in our case thermal energy) to enable it to surmount the potential barrier hindering such motion. Two distinct possibilities arise: (i) the molecule may rotate about one of its symmetry axes or, (ii) the amino-group may rotate about the C-N bond joining it to the phenyl ring.

Gutowsky and Pake<sup>5</sup> have shown that the rotation of the molecule as a whole reduces the value of  $S_j$  to a value  $(3 \cos^2 \gamma_{jk} - 1)/4$  times the rigid lattice value where  $\gamma_{jk}$  is the angle between the line joining the nuclei  $j$  and  $k$  and the axis of rotation. The reduced value of the intramolecular contribution comes out to be  $2.19 \text{ gauss}^2$  for the rotation of 2-5 dichloroaniline.

Andrew and Eades<sup>6</sup> have shown that the rotation of the molecule about one of its symmetry axes reduces the inter-molecular contribution by a factor of 0.24 yielding a value of  $2.60 \text{ gauss}^2$  for the sample under investigation.

The total rotational second moment is, therefore,  $2.19 + 2.60 = 4.79 \text{ gauss}^2$ . This value falls too short of the experimental value of second moment ( $16.4 \text{ gauss}^2$ ) at about  $360^\circ \text{K}$  and therefore, the possibility of the rotation of the 2-5 dichloroaniline about one of its symmetry axes may safely be ruled out.

The crystal structure study of 2-5 dichloroaniline indicates that the lattice is bereft of any hydrogen bonding between the different substituents. Further the dielectric studies of Kramer<sup>7</sup> reveals that the substitution of the chlorine atoms has little effect on the amino-group. This leads to the conclusion that there is a distinct possibility of the rotation of the amino-group about the C-N bond. The reduction in the value of the second moment due to the free rotation of the  $\text{NH}_2$  group taking the N-H bond lengths as  $1.015 \text{ \AA}$  and following the arguments given in a previous publication<sup>8</sup> should be  $19.26 \times 3/4 \times 2/5 = 5.78 \text{ gauss}^2$ . The reduced value of the second moment due to the rotation of the amino-group should, therefore, be  $23.53 - 5.78 = 17.75 \text{ gauss}^2$ . This value compares favourably, within the limits of experimental error, with the experimental value  $16.40 \text{ gauss}^2$  at  $320^\circ \text{K}$ . Hence we conclude that it is possible for the amino-group in solid 2-5 dichloroaniline to execute free rotation about the C-N bond at temperatures higher than the room temperature.

The second moment remains nearly the same as the melting point of the sample is approached. Hence it may be concluded that no other type of molecular motion takes place in the sample.

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# THERMAL EMISSION SPECTRUM OF THE MOLECULE—MgBr

WHILE Olmsted<sup>1</sup> was probably the pioneer to observe band spectrum of the molecule—MgBr in emission from a Bunsen Flame, Walters and Barrett<sup>2</sup> were the first to investigate it in absorption. These bands appeared in the violet region and were degraded to the shorter wavelength side. Later on, Morgan<sup>3</sup> also observed these bands in more extended form and proposed satisfactory vibrational analysis on the basis of the electronic transition as  $A^2\Pi-X^2\Sigma$ . In 1942, Harrington<sup>4</sup> recorded quite extensive data of absorption bands for this molecule and analysed them to form two separate systems lying in the spectral region  $\lambda\lambda$  3806–3991 and  $\lambda\lambda$  2548–2646. Yamdagni<sup>5</sup> restudied the absorption spectrum due to the molecule—MgBr and reported about ten additional bands degraded to shorter wavelength side in the spectral region  $\lambda\lambda$  3660–3790 but in view of the fact that these bands were weak and diffuse, no vibrational assignments could be made. Recently, Patel and Patel<sup>6</sup> carried out the rotational structure study for the (0,0) band of the A—X system and confirmed the relevant electronic transition to be  $A^2\Pi-X^2\Sigma$ .

The present communication presents an account of the authors' findings about  $A^2\Pi-X^2\Sigma$  system of the molecule—MgBr. The spectrum has been excited for the first time using thermal emission technique and quite a large number of new bands have been recorded and analysed.

A small quantity of pure solid magnesium bromide was put in the experimental tube of the high temperature graphite tube furnace. The spectrum has been excited at a temperature of about 2,200° C in the atmosphere of argon. An exposure of about ten minutes was found sufficient to obtain nice photographs on HP-3 panchromatic plates employing Hilger E-492 large quartz spectrograph. Copper arc provided the reference lines.

**Results and Discussion.**—The  $A^2\Pi-X^2\Sigma$  system consisting of bands degraded to shorter wavelength side of the molecule—MgBr known in the spectral region  $\lambda\lambda$  3806–3991 has been recorded in the extended form in the spectral region  $\lambda\lambda$  3659–4033. All the four heads could not be distinctly identified due to low dispersion of the spectrograph but their Q heads which are found to be overlapped on the shorter wavelength side may be nicely represented by the relation,

$$\text{System } A_1-X, v_{c_2} = 25766.85 + 393.91 (v' + \frac{1}{2}) - 2.04 (v' + \frac{1}{2})^2 - 374.14 (v'' + \frac{1}{2}) + 1.47 (v'' + \frac{1}{2})^2.$$

$$\text{System } A_1-X, v_{c_2} = 25877.00 + 394.00 (v' + \frac{1}{2}) - 2.00 (v' + \frac{1}{2})^2 - 373.40 (v'' + \frac{1}{2}) + 1.20 (v'' + \frac{1}{2})^2.$$

The wavelength of the bands with their corresponding wavenumbers in vacuum and vibrational assignments are presented in Table I.

TABLE I  
Band heads of the system  $A^2\Pi-X^2\Sigma$  for the molecule—MgBr

$\lambda_{air}$ in Å	$\nu_{vac}$ in $cm^{-1}$	Analysis ( $v', v''$ )
3659.5	37318	$Q_1 (-1, 0)^*p$
3697.2	27040	$Q_2 (3, 0)^*p$
3711.2	26938	$Q_1 (3, 0)^*p$
3745.8	26689	$Q_2 (-1, 2)^*p$
3748.2	26672	$Q_2 (3, 1)^*p$
3749.9	26660	$Q_2 (2, 0)^*p$
3761.7	26576	$Q_1 (-1, 2)^*p$
3763.9	26561	$Q_1 (3, 1)^*p$
3765.4	26550	$Q_1 (2, 0)^*p$
3805.5	26270	$Q_2 (1, 0)$
3821.2	26162	$Q_1 (1, 0)$
3862.1	25885	$Q_2 (0, 0)$
3870.5	25829	$Q_1 (3, 3)$
3873.2	25811	$Q_1 (2, 2)$
3876.4	25790	$Q_1 (1, 1)$
3878.6	25775	$Q_1 (0, 0)$
3918.4	25513	$Q_2 (0, 1)$
3921.7	25492	$Q_1 (5, 6)^*p$
3923.4	25482	$Q_1 (4, 5)^*p$
3926.0	25464	$Q_1 (3, 4)^*p$
3929.1	25444	$Q_1 (2, 3)$
3932.0	25425	$Q_1 (1, 2)$
3935.1	25404	$Q_1 (0, 1)$
3976.7	25139	$Q_2 (0, 2)$
3982.3	25104	$Q_1 (3, 5)^*p$
4033.1	24788	$Q_2 (0, 3)^*p$

\* New bands observed by the authors.

p Heads are involved in the perturbation and predissociation effect and are diffuse.

The analysis is found to be well in agreement with the one proposed by Harrington<sup>4</sup>. The  $Q_2$  heads apparently converge producing thereby a wide background in  $\Delta v = 0$  and 1 sequences. This is probably due to perturbation and predissociation effect as also pointed out by Harrington<sup>4</sup>. Due to the same effect the  $Q_1 (4, 4)$  and  $Q_1 (5, 5)$  bands are very weak and diffused and are not measurable, while the bands upto  $v = 3$  quantum number of  $\Delta v = 0$  sequence of the  $A_1-X$  system are found to be sharp.

The weak and diffuse bands as reported by Yamdagni<sup>5</sup> and suggested by him to constitute a new system could not be photographed in our experiment. However, his intense bands of the new system were no doubt observed and on precise measurement were found to fit well in the vibrational analysis of the  $A_1-X$  system as (2, 0) (3, 0)

and (4,0) heads. These bands are also involved in perturbation and predissociation phenomenon.

Since, thermal emission is a low energy excitation process, the C-X band system lying in the ultraviolet region could not be excited.

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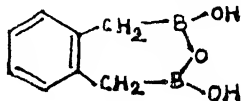
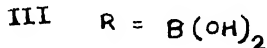
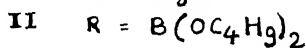
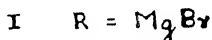
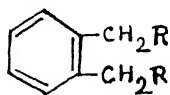
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### *o*-XYLYLENE DIBORONIC ACID AND ITS ANHYDRIDE

MICHAELIS *et al.*<sup>1</sup> obtained phenyl-boronic acids by hydrolysing the aryl boron chlorides; while Khotinsky and Melamed<sup>2</sup> obtained arylboronic acids by adding ethereal solutions of tri-alkyl borates to aryl magnesium bromide and hydrolysing the reaction product. Bean and Johnson's method<sup>3</sup> consisted of adding Grignard reagent to tri-alkyl borate at  $-80^{\circ}\text{C}$  and hydrolysing the product. Musgrave and Park<sup>4</sup> modified Bean and Johnson's method to get a better yield by heating under reflux the mixture of aryl halide, alkyl borate and magnesium turnings.



IV

*o*-Xylylene diboronic acid, a new diboronic acid, was prepared by this modified procedure<sup>4</sup>. Use of freshly cut pieces of magnesium ribbon and tetrahydrofuran solvent facilitates the reaction. The

Grignard reagent could be prepared at  $60-65^{\circ}\text{C}$  and addition of tri-butyl borate may be carried out at ice-salt bath temperature or at room temperature, thus avoiding the use of very low temperature ( $-80^{\circ}\text{C}$ ).

(1) *Preparation of o-xylylene diboronic acid.*— Freshly cut pieces of magnesium ribbon (0.5 g), dry tetrahydrofuran (50 ml), *o*-xylylene dibromide<sup>5</sup> (2.64 g) and tri-*n*-butyl borate (4.6 g) and a crystal of iodine were refluxed on an oil bath. After refluxing for 8 hours, THF was distilled off. The residue was treated with sodium hydroxide (10%) solution, warmed, and then steam distilled to remove butyl alcohol. The hot residue was filtered. The filtrate was cooled, acidified with dilute hydrochloric acid, and extracted with ether. The ethereal extract was washed with water and dried (sodium sulphate). *o*-Xylylene diboronic acid (0.78 g, 40%, m.p.  $144^{\circ}\text{C}$ ) was obtained as colourless flakes when ether was removed *in vacuo*.

Found, B : 11.2.  $\text{C}_8\text{H}_{10}\text{O}_4\text{B}_2$  requires, B : 11.34%.

(2) *Dehydration of o-xylylene diboronic acid.*— *o*-Xylylene diboronic acid (0.25 g) was taken with dry toluene in a azeotropic distillation unit and distillation continued for 4 hours. Toluene was distilled off under reduced pressure. *o*-Xylylene diboronic acid anhydride was obtained (0.24 g) in colourless powder form, m.p.  $141^{\circ}\text{C}$ .

Found, B : 12.4.  $\text{C}_8\text{H}_{10}\text{O}_3\text{B}_2$  requires, B : 12.5%.

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### DAMAGE CENTRES IN IRRADIATED POTASSIUM BROMATE

EARLIER work<sup>1</sup> has shown that annealing in irradiated potassium bromate is a combination of a unimolecular and a bimolecular process which is indicative of the existence of two groups of damage centres in the irradiated substance. Confirmation for this has been obtained from isochronal annealing and linear tempering studies.

Potassium bromate was irradiated with Co-60  $\gamma$ -rays to a dose of 50 Mrad at a dose rate of 0.83 Mrad/hr. For isochronal annealing, samples of the irradiated crystals were heated at various

temperatures in the range 100–300°C at 10°C intervals for 5 hr at each temperature. In linear tempering, heating was done at a constant rate of 4°C/min to increasing temperatures; samples were withdrawn after every 10°C rise of temperature. The same analytical procedure<sup>2</sup> as already described was followed.

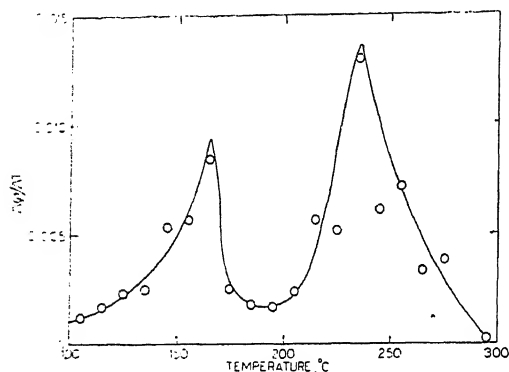


FIG. 1. Linear tempering in gamma-irradiated potassium bromate.

Data for linear tempering are shown in Fig. 1 on a plot of  $\Delta\phi/\Delta T$  against temperature;  $\Delta\phi$  is the increase in the fraction of the damage annealed over an increase  $\Delta T$  in temperature. Two peaks occur which show the presence of two groups of damage centres in the irradiated substance. A similar curve is obtained for isochronal annealing.

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#### SEPARATION AND IDENTIFICATION OF OPIUM ALKALOIDS BY THIN LAYER CHROMATOGRAPHY

OPIUM is an air-dried juice obtained by incision of the unripe capsules of the white poppy<sup>1</sup> (*Papaver somniferum* Linn.) belonging to family Papaveraceae and is grown in India, Persia, Asia Minor, Turkey, China and Egypt, also cultivated in England and other cold countries like Russia and Yugoslavia. Poppy capsules, when ripe and dry are more or less rounded, irregularly formed or flattened masses, weighing from 250 to 1000 gm. Opium is a dark brown solid possessing a characteristic odour.

Opium is a highly complex body, containing 25 alkaloids, combined with meconic, lactic and sulphuric acids<sup>2</sup>. Of these the most important is morphine which occurs in combination with meconic acid. Next in importance are codeine, narcotine, papaverine and thebaine. In addition to these, there are 3 neutral components and 10 heterocyclic nitrogenous compounds which do not occur in the fresh latex but are found as a result of oxidation, hydrolysis and recombination. The percentage of the constituents varies with the country. Indian opium yields from 9.5 to 14% of morphine, 1.8 to 4% of codeine, 3.0 to 7.6% of narcotine, 1% of papaverine and 0.5 to 1% of thebaine<sup>3</sup>.

In India the cultivation of the poppy is permitted only under a licence and that too now is practically limited to U.P. and the Government purchases the whole product.

A large number of alkaloids have been chromatographed from opium by various forms of chromatography. According to U.S. Treasury Department these alkaloids are present in opium either as their sulphate or as their meconate but not as their free base<sup>4</sup>. In the present study, thin layer chromatography for the separation and identification of opium alkaloids is used.

#### EXPERIMENTAL

##### Material and Methods

- (1) Silica Gel G. (E. Merck Germany).
- (2) Thin layer chromatography apparatus.
- (3) Glass plates, strips and thin-walled micro-capillary tubes.
- (4) Solvent systems :
  - (A) Benzene : Dioxane : Ethanol : Ammonium Hydroxide 10 : 8 : 1 : 1.
  - (B) Benzene : Dioxane : Potassium hydroxide 40% : Absolute Alcohol 56 : 40 : 2 : 2.
  - (C) Ethyl acetate : Methanol : Ammonium Hydroxide (Con.) 17 : 2 : 1.

All reagents are prepared from analytical reagent grade chemicals.

- (5) Spray reagent.

**Dragendorff's Reagent.**—1.3 gm of the bismuth nitrate in 60 ml of water and 15 ml of glacial acetic acid were added to 12 gm of potassium iodide in 30 ml of water. The mixture was diluted with 100 ml of water and 25 ml of glacial acetic acid.

**Preparation of Chromato-Plates.**—The method of thin layer chromatography devised by Stahl and Kaltenbach<sup>5</sup> was employed. Glass plates were coated with a thin layer of silica-gel prepared from a slurry consisting of silica-gel G 30 gm, demineralised water 55 ml and methyl alcohol 5 ml.

TABLE I

Sl. No.	Substances	$R_f \times 100$ with solvent systems			UV Examination	Colour of spot with Dragendorff's Reagent
		A	B	C		
1.	Opium Extract i ..	36	42	49	Light grey	Orange
	ii ..	64	61	69	Yellowish	Red
	iii ..	90	90	92	Bluish	Pinkish red
	iv ..	97	98	98	Yellowish brown	Pink
2.	Morphine ..	36	42	49	Light grey	Orange
3.	Codeine ..	64	61	69	Yellowish	Red
4.	Papaverine ..	97	98	98	Yellowish brown	Pink

The plates were allowed to dry for 15 minutes and then placed in an electric oven for 30 minutes at 110° C.

**Extraction of Alkaloids from Opium.**—2 gm of the sample was boiled with 5 ml of distilled water for few minutes, cooled and filtered. The filtrate was tested for the presence of meconic acid and morphine with neutral ferric chloride, hydrochloric acid and Marquis's test respectively. The positive test confirmed the presence of alkaloids.

**Method.**—Small aliquots of the filtrate were transferred to the thin layer plates with the help of thin capillary pipettes. Pure sample of morphine, codeine and papaverine were also individually chromatographed alongside the opium extract for comparison purpose. Then the plates were inserted into the developing tanks each containing 100 ml of the developing solvent. The developing tanks were made airtight by covering with their lids. The plates were then removed from the tanks after the solvent had moved about 10 cm distance from the point of application of the spots. The plates were then dried and examined under UV light for fluorescence and then sprayed with spray reagent. The  $R_f$  values of the spots, U.V. fluorescence given by the alkaloids and the colour of the spots are given in Table I. The opium alkaloids and alkaloids spotted for comparison were developed with solvent system A, B and C.

**Discussion.**—The alkaloids of opium were separated by developing with three different solvent systems. Opium extract resolved into four spots with each of the three solvents which were identified by comparing their  $R_f$  value, UV fluorescence and the colour of the spots with known alkaloids which were spotted alongside of the opium alkaloids. Each solvent system yielded different  $R_f$  values. On comparison, it was found that spot

No. i is that of morphine. No. ii of codeine and No. iv is that of papaverine. Spot No. iii gave blue fluorescence under UV light and pinkish red colour when sprayed with Dragendorff's reagent, due to lack of other alkaloids for comparison, spot No. iii could not be identified positively. The observations and  $R_f$  values of opium alkaloids and alkaloids used for comparison are given in Table I.

The authors wish to thank UGC for giving a grant to the scheme and also express their gratitude to Professor D. P. Jatar, Head of the Department of Criminology and Forensic Science and the Dean of the Faculty of Science, for the kind encouragement throughout the work.

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#### DOLOMITISATION OF BILARA LIMESTONE OF TRANS-ARAVALLI VINDHYAN BASIN DURING EOCENE PERIOD

The calcareous member of Trans-Aravalli Vindhyan basin, known as Bilara limestone, occupies an area of about 2500 sq. km in western Rajasthan. It is strikingly characterised by vague stratification,

collapse breccia structures, stony-waste appearance, and irregular dips in all directions, often at high angles. The limestone is mostly dolomitic in composition, fine grained, non-crystalline and at places is cherty and siliceous. Few scattered pockets of high grade limestone, suitable for lime industry, are observed within this vast stretch of dolomitic limestone. The author had reported occurrence of algal stromatolites in Bilara limestone indicating shallow water deposition in the intertidal or near the intertidal zones<sup>1</sup>.

Detailed study recently made by the author has further revealed that majority of stromatolites have pebbles of chert and silicified limestone as nucleus around which the algal structures have developed. The chert pebbles have been derived from the chert found associated with the Bilara limestone. It is therefore concluded that these organic-sedimentary structures are younger in age than the Bilara limestone.

The exploratory drilling operations, recently carried out by the Ground Water Department, have revealed the occurrence of lignite at Indawar (26° 34' 30" : 73° 56') about 18 km south of Merta Road, and near the limestone exposures. The lithologic log of the bore-hole shows a distinct correlation with the Palana (Bikaner) bore-holes. The lignite has been encountered at the same depth followed by earthy brown shales (Fullers earth) at a depth of 210 metres. The lignite at Palana has been found to be associated with white to buff limestones and shales with characteristic fossils belonging to the genera of *Nummulites* and *Assilina*<sup>2</sup>. It has accordingly been assigned the epoch of Laki series of Eocene period. With the occurrence of lignite at Indawar, it is proved beyond any doubt that the area on the east and north of Bilara limestone was surrounded by sea during Eocene period with estuaries towards the land. The intensive dolomitisation of Bilara limestone has taken place during the Eocene period when magnesium was provided by the sea water. Dolomitisation had resulted in the reduction of volume by 14% causing collapse structures and breccia. It was during this period that the shallow water conditions prevailed necessary for the growth of algal structures.

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### THE ALGAL STRUCTURE AND PHOSPHORITE IN THE ARAVALLI ROCKS OF JHABUA DISTRICT (M.P.)

In the Archaean terrain of Jhabua District, Madhya Pradesh, phosphorite occurrences were discovered in March 1973, in a dolomitic limestone-chert bed about 12 km long showing stromatolitic structures, between Amlamal (23° 00' : 74° 25') and south of Rambhapur (22° 55' : 74° 30') villages. Of the number of phosphorite bodies discovered the ones near Khatamba, Kelkua and Piproda villages are very significant from economic aspects.

The limestone-chert member is a part of the Aravalli Group of rocks comprising quartz phyllites, chlorite-quartz phyllites, quartzites, and limestone bands. The metasediments are regionally folded into plunging open anticlines and closed synclines which are overfolded towards east. The limestone-chert bed forms part of the folded eastern limb of a major syncline whose western limb is represented by predominantly chert outcrops forming the NNW-SSE trending hill ranges near Padtala-Gwali-Dhebar villages. Extensive feldspathisation affecting the quartzitic and phyllitic rocks has given rise to granitoid and gneissose rocks.

The dolomitic limestone is very finegrained, massive and contains sparsely distributed clastic quartz grains. The chert bands occur as lenses and intercalated bands within the limestone. Discontinuous lenses and bands of phosphatic bodies occur within the chert and limestone beds. The phosphate is dark bluish grey in colour and the rock phosphate analyses upto 37%  $P_2O_5$ . The phosphatic matter, probably collophane and allophane, is associated mostly with the stromatolitic structures seen in chert as well as in limestone. The association of stromatolites and phosphorite, however, is not ubiquitous. Thin laminae of dark bluish grey phosphate minerals alternating with thicker laminae of limestone showing penecontemporaneous deformational structures are seen. Phosphate bodies as near Piproda village are without any associated stromatolites. The precipitation of phosphate, therefore, was not directly dependent on the action of algae, but such close association emphasises similarity of environment required for both.

The stromatolites of both chert and in limestone bodies are almost all broken and laid irregularly, but generally parallel to bedding. The degree of fragmentation is variable and at places they are partly well preserved. The stromatolites are generally of the columnar type and oncolites. Following Cloud and Semikhatov (1969), the form genera of columnar varieties identified indicate that *Conophyton* is by far the most predominant Group

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followed closely by Colonella. Bicalia and Kusiella, though present, are uncommon. Under microscope the stromatolites show their laminae to be composed of fine aggregates of carbonates, chert and collophane. The interstitial material is composed of fine detrital grains of carbonate and quartz. The Conophyton-Colonella assemblage, according to Raaben (1969) is indicative of possible Lower Riphean (1600-1000 m.y.) age. This, however, cannot be confirmed in case of these Aravalli beds without further work. On the basis of available radiometric data, Sarkar (1972, pp. 261-62) concluded that Aravalli orogeny is dated ~ 2000 m.y. which means that Aravalli sediments are very much pre-Riphean. The data now available, however, are insufficient to come to any firm conclusion.

Logan *et al.* (1964, pp. 77-81) have suggested an environmental significance of the stromatolite structures. Others (*cf.* Walter and Preiss, 1972, p. 92) agree with this suggestion. The presence of SH-type stromatolites and oncolites indicates, as observed by Logan *et al.* (1964, pp. 79-81), an exposed intertidal environment for the limestone-chert and, therefore, also phosphorite deposits. The orthoquartzite-limestone assemblage of the Aravalli metasediments, their inorganic depositional structures like current bedding, and the various precontemporaneous deformational structures indicate their shallow water origin on shelf with low relief of the borderlands. The preponderance of broken stromatolites, high frequency of intraformational chert—and limestone-breccia—point out exposed inter-tidal environment where periodic high energy wave action predominated.

The phosphate was deposited in the same environment when influx of clastic sediments had almost ceased. But whether any bathyal condition prevailed in the adjacent part of the ancient ocean as required for the supply of phosphate according to Kazakov (1937), as quoted in Reeves and Saadi (1971) is yet unknown.

Geological Survey of  
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#### TURBIDITE STRUCTURES FROM A SANDSTONE-SHALE SEQUENCE OF TALCHIR GROUP, TALCHIR BASIN, ORISSA

SOME turbidite structures are described from a sandstone-shale sequence of Talchir Group, Talchir-basin, around Angul (Long. 85° 2'-85° 6'; Lat. 20° 52'-20° 54'), Dhenkanal District, Orissa. Similar structures have also been described from the Talchir Group in other parts of India<sup>1-3</sup>. The lithic unit is 2-6 meters thick and exposed in a NE-SW direction for a distance of about 1.5 Km. It rests on the sandstones of Boulder Conglomerate Complex<sup>4</sup> and grades in the down-dip direction (north) into a thick sequence of interbedded siltstone-needle shale-marlstones.

The lithic unit can be differentiated into two types, *viz.* sheet phase and channel phase. The sheet phase consists of even-bedded, laterally persistent, purple, medium to finegrained sandstones, 1.00 cm-25 cm thick, separated by gray to dark laminated shales. The channel phase sandstones are lenticular, elongate down-dip and merge into laterally equivalent sheet sandstones. They are characterised by a lag conglomerate at the base followed by about 2.5 m thick trough cross-stratified coarse sandstones.

The sheet phase sandstones show various bedding types such as graded bedding, horizontal laminations, rippled drift cross-laminations of the types A, B, C<sup>5</sup> and massive bedding. Also combination of bedding types occur in succession one above the other in a single unit identical to turbidite sequences described by Bouma<sup>6</sup>. The sandstones show sole marks like flute casts, furrow casts<sup>7</sup>, rill casts, load modified longitudinal furrows and ridges<sup>8</sup>, load casts and groove casts. The bedding plane of the sandstones shows asymmetric and interference ripple marks. The shales are marked by parallel laminations.

Rocks belonging to Talchir Group are thought to be of glacio-fluvial and glacio-lacustrine origin. Lithological character, geometry and combination of sedimentary structures suggest a predominantly lacustrine origin of the lithic unit. Association of channel sandstones suggests it to be a lake marginal facies<sup>9</sup> deposited probably along a lake shelf where the glacial rivers were debouching into the lake. The sedimentary structures may be the product of

turbidity currents triggered due to melt water flash floods diving into the lake water. Kuenen<sup>10</sup> has shown that turbidity currents may form under such conditions.

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**EFFECT OF INFESTATION OF LIPAPHIS  
ERYSIMI (KALT.) (APHIDIDAE, HOMOPTERA)  
ON QUALITATIVE AND QUANTITATIVE  
CHARACTERS OF SEEDS OF MUSTARD  
(BRASSICA JUNCEA COSS)**

YIELD losses in Brassicas due to the mustard aphid, *Lipaphis erysimi* (Kalt.), are reported in the literature<sup>2-4</sup>. Though the seed quality as well as quantity determines the total output of the crop, there appears to be no study so far made on the cumulative effect of aphid infestation on the qualitative and quantitative composition of seeds.

of rai (T-59), a commercial variety of *Brassica juncea* Coss, due to aphid infestation throughout the cropping season.

Affected shoots of different plants exposed to various degrees of aphid infestation under natural condition were collected at random during harvest. Seeds of such plants were graded according to their thousand seed weight in the following manner:

- Grade 1 : Above 5 g (mean 5.340 g).  
Grade 2 : Between 4 and 5 g (mean 4.321 g).  
Grade 3 : Between 3 and 4 g (mean 3.461 g).  
Grade 4 : Between 2 and 3 g (mean 2.521 g).  
Grade 5 : Below 2 g (mean 1.179 g).

Seeds of 20 different shoots belonging to same grade were mixed together and their sizes were determined by passing them through sieve of different meshes. The viability was tested in seed germinator. The composition of seeds, viz., oil, iodine value of oil, protein, total sugar, allyl isothiocyanate, ash and moisture, of the above grades were determined by the standard procedures of AOAC<sup>1</sup>. The results are given in Tables I and II.

TABLE I

Size of mustard seeds of different grades  
(based on 1,000 seeds, mean of 4 replicates)

	Above 2.0 mm	2.0-1.5 mm	1.5-0.75 mm	Below 0.75 mm
Grade 1 ..	963 (5.240)	37 (0.110)	Nil	Nil
„ 2 ..	741 (3.875)	259 (0.930)	Nil	Nil
„ 3 ..	210 (1.295)	612 (2.065)	162 (0.082)	16 (0.003)
„ 4 ..	35 (0.175)	710 (1.925)	240 (0.315)	15 (0.002)
„ 5 ..	Nil	268 (0.540)	540 (0.495)	192 (0.050)

Figure in parenthesis gives the corresponding mean weight in g.

TABLE II

Viability and chemical composition of seeds of rai expressed in per cent (mean of three replicates)

	Viability	Oil	Iodine value	Protein	Total sugar	Allyl isothio- cyanate	Ash	Moisture
	1	2	3	4	5	6	7	8
Grade 1 ..	98	37.40	101.3	20.05	3.49	0.26	4.51	4.15
„ 2 ..	95	32.60	102.0	20.12	3.55	0.28	4.72	4.40
„ 3 ..	64	29.10	101.0	22.18	3.73	0.32	4.84	4.60
„ 4 ..	56	24.06	102.9	26.28	3.96	0.42	5.30	5.41
„ 5 ..	25	19.96	100.7	26.88	5.08	0.48	5.68	6.94
S.Em ±	2.24	1.45	2.62	0.19	0.09	0.017	0.036	0.05
C.D. at 5% level ..	5.00	3.23	NS	0.44	0.20	0.04	0.08	0.11

The present study was undertaken at the Pulses and Oilseeds Research Station, Berhampore, West Bengal, to determine the change in seed character

It is evident from Table I that depletion in seed weight occurs with the corresponding decrease in seed size. Since it was already known that aphid

infestation brings down the yield of mustard to an extent of about 70.2 to 91.3%<sup>2-4</sup>, the different grades of seed weight represent the various extent of aphid damage. Table II shows that the seed weight has a direct bearing on chemical composition. In oil yielding crops like mustard bolder and heavier seeds contain more oil. Such direct correlation of seed size and oil content has been known in brown sarson<sup>5</sup>. Viability of seeds is also dependent on seed size. Non-significant variation in iodine value of the oil of different grades suggests that the percentage of unsaturated fatty acids has not been altered due to infestation. The smaller seeds having large proportion of seed coat, rich in fibre, account for their higher ash as well as total sugar content than their bolder counterparts. Allyl isothiocyanate which is known to be a specific substance giving stimulus for host selection by aphid<sup>6</sup>, has been increased in the lighter seeds, with a corresponding increase in the total sugar content.

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#### A NEW BACTERIAL BLIGHT DISEASE OF *MURRAYA KOENIGII* SPRENG.

*Murraya koenigii* Spreng., a member of the *Rutaceae*, is commonly grown in private gardens in western India. In September–October of 1973, a blight disease was noticed on this plant at Bhavnagar, Gujarat State.

The disease at first appears on first formed leaves as numerous, irregular, water soaked, brown to black spots mostly on the margin, measuring from 1 to 2 mm in diameter. In severe cases, the pathogen attacked the entire leaf causing blighting of it.

The causal bacterium was isolated in pure culture on potato dextrose agar by usual pour plate and streak plate methods. On artificial inoculation of young and mature plants of *M. koenigii*, the typical symptoms developed in about fifteen days (Fig. 1). The pathogen was reisolated in pure culture and identified with the original.



FIG. 1. Symptoms on leaves from artificially inoculated mature plants.

By artificial inoculation, the bacterium could not infect *Tamarindus indica* L., *Triticum vulgare* H., *Capsicum annuum* L., *Banlinia recemosa* L., *Gossypium* spp., *Lawsonia alba* L., *Sorghum vulgare* P., *Carissa carandus* L., *Phaseolus* spp., *Citrus* spp., while infect the second member of the *Murraya* genus, *M. exotica* within twenty-five days.

The morphological, cultural and physiological characteristics of the pathogen showed it to belong to the genus *Xanthomonas*.

Since no *Xanthomonas* disease is reported from the host from anywhere and as host-specificity is considered as accepted criterion for speciation in the genus *Xanthomonas*<sup>1-3</sup>, it is, therefore, proposed to designate the pathogen as *Xanthomonas murrayae* nov. sp. The technical description of the pathogen is as follows:

#### *Xanthomonas murrayae* Nov. Sp.

Short rods with rounded ends, usually single, rarely in pair, measuring  $1-1.9 \times 0.5-0.8$  microns, gram-negative, motile with polar flagellum, capsulated, no endospore, non-acidfast, colonies on



PDA plate—big, circular, smooth surface and entire margin, butyrous and glistening yellow. Growth on PDA slant is abundant, filiform, convex, smooth opaque, butyrous and glistening yellow, the medium remains unchanged. On nutrient agar growth is moderate, filiform, flat, smooth, opaque and glistening yellow.

Starch hydrolyzed, casein digested, tributyrin hydrolyzed, gelatin liquefied, milk peptonised and litmus reduced, ammonia and hydrogen sulfide produced, indole not produced, V.P. and M.R. tests negative, nitrate not reduced to nitrite, uric acid utilized but not citrate, growth retarded by 2% and suppressed by 3% sodium chloride. Produce acid without gas from glucose, sucrose, galactose, xylose, fructose, ribose, mannitol, glycogen but no acid and gas from lactose, rhamnose, inositol and inulin. Catalase positive, strictly aerobic, optimum temperature 27°–30° C, thermal death point 55° C.

The bacterium is pathogenic only to *Murraya* plants producing blighting of leaves.

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#### THE OCCURRENCE OF RACE 5 OF *XANTHOMONAS MALVACEARUM* IN INDIA

SEVENTEEN physiologic races have been distinguished in the pathogen of the bacterial blight of cotton, *Xanthomonas malvacearum* (E.F.Sm.) Dows.1,2. Of these, five, viz., races 3, 10, 14, 16 and 17 have been reported to be present in India. Race 10 is ubiquitous and occurs in all parts of India wherever cotton is grown. Recently while examining collections of the pathogen occurring on different varieties in the germplasm, one isolate purified out of a mixture occurring on variety PRS 72 was pathogenic to Acala 44, Stoneville 2B–S9, 1–10 B and 20–3 but did not affect the other differential varieties of Hunter *et al.*<sup>1</sup>. This isolate thus corresponds to race 5 described by these authors. It has been found to be highly pathogenic and to

affect a wide range of varieties belonging to *Gossypium barbadense* and *G. hirsutum* including Sujata, MCU-1, MCU-5, Laxmi and P1 D-40.

A synergistic effect was observed between race 5 and race 10. While the two races when inoculated separately on leaves of variety Laxmi gave rise to angular leaf spots which were 3 to 5 mm across, a mixed inoculum containing approximately equal number of bacterial cells per unit volume of inoculum fluid gave rise to angular spots which were 6 to 9 mm in diameter. In addition, the spots appeared about two to three days earlier with the mixed inoculum, i.e., after 8 days while the inocula of the two races used separately gave rise to spots after 10 to 11 days. However there was no increase in the number of spots. Similar results were obtained by introducing the inoculum into the veins or petioles or vascular elements of young plants, either by injecting with a hypodermic syringe or by pricking with a fine needle through a drop of the bacterial suspension placed on the surface by means of a fine nylon headed steel entomological pin. The linear vein lesions in this case appeared 10–11 days after inoculation and were 10–14 mm long with the pure races, but appeared after 8 days and the lesions were 20 to 23 mm long with a mixed inoculum.

Comparable results were obtained with varieties MCU-5 (*G. hirsutum*) and Sujata (*G. barbadense*). However both the individual races and a mixture could bring about only small lesions (Grade 2) on variety K-7 (*G. arboreum*), and they could not infect other known resistant varieties like 101–102B, BIA 592, P.14–T.128 and Reba-B-50.

It would appear that in epiphytotic years when cotton crops suffer severe damage, such mixtures of races possessing synergistic virulence may be involved. The occurrence of this phenomenon will also complicate the task of breeding for bacterial blight resistance.

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# A VIRUS CAUSING LEAF DISTORTION IN *OXALIS CORNICULATA* L.

*Oxalis corniculata* L. vernacular "Khatti—Mithi Belori", is a common weed in fruit orchards at Simla, Himachal Pradesh. During July-August, 1972 symptoms of vein enations and distortion accompanied with crumpling were noticed on the leaves of naturally affected plants (Fig. 1).



FIG. 1. Diseased plant of *O. corniculata*.

Later surveys revealed that the disease was patchily distributed with pockets of infected plants. The disease incidence varied from 20 to 25% at Flowerdale Estate and at other fruit orchards inspected. The disease syndrome was found to be graft-transmissible and thus proving its virus nature. Further investigations provided additional results which are reported herein.

Under field conditions the virus primarily affects the foliage. The leaves of affected plants develop veinal enations distortion and crumpling of the leaflets. In severely infected cases the veinal enations become more conspicuous. The leaf remains green. Foliar symptoms on very young leaves are very much pronounced. Stems, blossoms and fruits reveal no observable symptoms.

The virus was not transmitted by manual inoculation of infected sap to healthy plants when sap was extracted in phosphate buffer (pH 7), 2-5% nicotinic acid or 4% polyethylene glycol 4000, with carborundum 600 mesh or celite as abrasive. Under host range the virus was easily transmitted through grafting to *Oxalis corniculata* only within 20 to 25 days. Following plant species could not be infected through grafting: *Datura stramonium* L., *D. metel* L., *Nicotiana glauca* L., *Chenopodium amaranticolor* Coste and Reyn and *C. quinoa* Willd. The virus was not recovered from these by back inoculations onto *O. corniculata*. The aphid *Myzus persicae* Sulz. failed to transmit the virus from *O. corniculata* to *O. corniculata*. The virus was not seed borne.

Further work is in progress. The virus is considered to be a new record since so far the genus *Oxalis* has not been recorded as host of any plant pathogenic virus.

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## A NEW RECORD OF THE SPERMOGONIAL ASSOCIATION WITH THE DEVELOPING ASCOCARPS OF *PHYLLACHORA* *SORGHI* HOEHNEL

Tar spot of sorghum incited by *Phyllachora sorghi* has been found to be regularly appearing in the fields of Agricultural College, Dharwar, as noticed in 1972 and 1973. The disease was found to be fairly severe on some of the varieties like SB-301, 401, 402, 407, 412, 414, 427, 461, 471, 473, 505 and 1529. Amongst these, SB-461 was severely affected as seen by the innumerable tar spots completely covering the surfaces of young and old leaves. In the early stage of infection, the tar spots were in the form of linear streaks, which later developed into black erumpent, spherical stroma. Microscopic examination of the young stroma, i.e., linear streaks revealed the presence of acervuli-like spermatogonia completely filled with spermatia, which ooze out of the ostiole in mass. Such spermatogonia were observed to be constantly associated with the ascocarp initials as seen in both hand and microtome sections giving an indication of their possible role in the sexual reproduction of this Ascomycete. Such association and their role in effecting spermatization has been already worked out in detail by Ananthanarayanan<sup>1</sup> and Jagtap<sup>2</sup> in *Cyclothea kamatii* and *P. simplicicola*, respectively. So far there is no report on the association of spermatogonia with the ascocarps of *Phyllachora sorghi*; being a new report, a brief description of the spermatogonia and their association is presented here.

Linear streaks were black, shining, erumpent, amphigenous upto 1 mm long and 0.2 mm broad.

On the surface of streaks, minute, pin-head like structures were seen.



Figs. 1-3. Fig. 1. Spermogonium with spermatophores and spermatia. Fig. 2. Spermogonium on the upper surface of the ascocarp initial; ascogonial coil (Asco.) in the centre of ascocarp initial. Fig. 3. Spermogonium associated with the young ascocarp, note the uni- to multinucleate ascogonial cells (a).

The young developing infection spots were cut from leaves and fixed in F.A.A. After dehydration in alcohol and passing through alcohol-xylene series, paraffin embedding was done following Sass<sup>3</sup>. The sections were cut at 5-7  $\mu$  thickness with the help of rotary microtome in cool hours. The

sections were stained with Heidenhain's hematoxyline using ferric-ammonium sulphate as mordant and fast destainer. Sections were mounted in Canada balsam and used for the observation. Hand sections mounted in lactophenol were also employed in the study.

Spermogonia (Fig. 1) were usually subcuticular amphigenous, ostiolate, conical and sometimes broad, upto 560  $\mu$  broad and 320  $\mu$  high. Spermatophores simple, hyaline, arranged in wall layers non-septate, 12-16  $\times$  1-2  $\mu$ . Spermatia oblong 1-celled, hyaline, uninucleate, 4-6  $\mu$  long and innumerable. They failed to germinate in water. Such spermogonia were seen in sections associated with the ascocarp initial having ascogonial coil (Fig. 2). Further developmental stages showed the differentiation of ascogonial coil into uni- to multinucleate ascogonial cells and such ascocarp initial was also constantly found associated with the spermogonia (Fig. 3). The sections from well developed spots revealed the presence of only mature perithecia and in none of the sections spermogonial association was observed. Further studies on the possible role of spermogonia in the sexual reproduction of *Phyllachora sorghi* and ascocarp development are in progress.

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#### A STATISTICAL ANALYSIS OF THORAX/HEAD LENGTH RELATIONSHIP IN THE INDIAN BLACK ANT, *CAMPONOTUS COMPRESSUS* FABR.

In *C. compressus* the logarithms of the length of the thorax and that of the head demonstrated a straight line relationship, showing that any increase in the logarithm of one parameter was associated with a direct increase in the logarithm of the other (Table I). This relationship can be expressed by the following logarithmic equation:

$$\text{Log } x = 0.3183 + 0.5808 \log y$$

where,  $x$  is the length of the thorax (mm), and  $y$  is the length of the head (mm).

TABLE I

Log length of thorax (mm)	Log length of head (mm)	Regression constants
0.5185	0.3617	
0.5911	0.4472	
0.5682	0.4698	
0.6021	0.4771	
0.6021	0.4771	
0.6021	0.4914	$a = 0.3183$
0.6021	0.5015	$b = 0.5808$
0.6812	0.6021	
0.6857	0.6021	
0.6812	0.6128	
0.6812	0.6128	
0.6857	0.6232	
0.6857	0.6232	
0.6990	0.6857	
0.6990	0.6902	

The relative difference in the lengths of the thorax and the head was higher in the specimens of smaller size-groups while in the larger individuals the growth of the thorax did not keep pace with that of the head. This was presumably due to the existence of positive heterogony in the head. This became evident from the decline recorded in the thorax/head ratio from 1.434 to 1.020, with advance in the linear dimensions.

A calculation of the heterogonic coefficients of the two parts shows that the heterogonic coefficient of the head (1.809) is considerably higher than that of the thorax (0.552). A positive heterogonic growth of the head of *Pheidole instabilis* has also been reported by Mayr *et al.*<sup>1</sup>.

For statistical evaluation of the intraspecific variations in the thorax/head relationship, the standard deviation of the thorax/head ratio for 14 degrees of freedom was calculated as 0.118 and the standard error as 0.030. The variance, the coefficient of variability and the level of significance (deviation) being 0.013, 9.624 and 0.786% respectively.

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# CARDIAC ACTIVITY DURING AESTIVATION OF THE SNAIL *PILA GLOBOSA* (SWAINSON)

THE Indian apple snail, *Pila globosa*, aestivates during summer, when the ponds and streams which they inhabit go dry<sup>1-3</sup>. During aestivation, remarkable physiological and biochemical changes occur which include loss of weight<sup>3,4</sup> and decrease in respiratory enzyme activities<sup>5,6</sup>, followed by other metabolic changes. These fundamental changes are supposed to be due to the presence of a steroid hormone in the cerebral ganglia, and a chloroform soluble factor in the digestive gland of aestivating snails<sup>7,8</sup>. The decrease in metabolic efficiency should be in consonance with the overall decrement in physiological activity of blood and circulation. Hence, it was felt desirable to study the pattern of heart beat in the aestivating snail, *Pila globosa*, and see whether the observed metabolic shift has any bearing on the cardiac activity.

The shells of normal and three months aestivated snails were opened with least injury, and the animal was kept in a petri dish containing *Pila* Ringer<sup>9</sup>, for 5 min at 35° C to recover from shock effects. The rate of heart beat in normal active snails was about  $26 \pm 2.9$  beats per min while in aestivated snails the rate dropped to  $13 \pm 0.7$  beats per min thus indicating about 53.7% decrement in the cardiac activity. Thus, one of the significant changes during aestivation is lowered circulatory efficiency of body fluids. The ATP-ase activity<sup>10</sup>, along with several oxidative enzyme activities, decreases<sup>5-7</sup>, indicating non-utilization or non-availability of the energy-producing systems needed for the physiological activity of organs such as the heart.

The addition of aestivated body fluid on the left pleurovisceral connective of normal snail decreased the electrical activity, while normal body fluid elevated the electrical activity of that connective in aestivated snail<sup>11</sup>. This is suggestive of the inhibitory effect of aestivated body fluids on the physiological activity.

Similar affinity was found to be under existence in aestivated snail hearts. Addition of aestivated body fluid reduced the rate of heart beat of normal snails by 50% ( $13 \pm 1.29$  beats per min) while the addition of normal body fluids on the hearts of aestivated snails resulted in about 70% increase in the rate of heart beat ( $22 \pm 2$  beats per min) (Table I). Hence, it may be presumed that aestivated and normal body fluids possess cardiac depressants and accelerators respectively in them.

Chromatographic analysis of body fluids of normal and aestivated snails showed a considerable increase in glutamic acid content during aestivation<sup>11</sup>. Glutamic and aspartic acids showed an inhibitory

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TABLE I

Effect of body fluids, selected amino acids and inorganic ions on the rate of heart beat in normal and aestivated snails. Rate of heart beat represented as number of heart beats/min  
Each value is an average of 6 individual analyses

Sl. No.	Name of test solution	Normal	't' test	Aestivated	't' test
1.	..	26±2.7	..	13±0.7	P>0.001
2.	Aestivated body fluid	.. 13±1.29	P>0.05	12±1	N.S.
3.	Normal body fluid	.. 24±3.0	N.S.	22±2	P>0.02
4.	Glutamic acid	.. 13±1.4	P>0.001	6±1.2	P>0.001
5.	Aspartic acid	.. 13±1.4	P>0.001	8±0.7	P>0.01
6.	Lysine	.. 26±1.4	N.S.	16±1.4	P>0.01
7.	Arginine	.. 24±2.46	P>0.05	15±0.7	P>0.001
8.	Calcium chloride	.. 22±1.9	P>0.05	10±1.4	P>0.01
9.	Magnesium chloride	.. 19±2.24	P>0.02	10±1.4	P>0.01

N.S. = Not significant.

influence on the electrical activity of the nervous system of both normal and aestivated snails, while lysine and arginine showed elevatory effects<sup>11,12</sup>.

In the light of glutamic acid inhibition of metabolism of aestivated snails, it was felt essential to study the effects of acidic and basic amino acids on cardiac activity.

The acidic amino acids, namely, the glutamic and aspartic acids induced 30–50% decrease in the rate of heart beat in normal and aestivated snails (Table I). The glutamic acid decreased the rate of heart beat of normal animal to the level of aestivated snail heart beat (Table I). Lysine and arginine had no effect on normal snails while they showed slight elevatory effect on aestivated snail hearts (25 to 33%). Calcium and magnesium induced slight inhibitory effect on both normal and aestivated snail hearts (Table I). Thus the variation in the cardiac activity could be not only due to change in the ionic concentration of body fluids<sup>13</sup>, and of neurosecretions<sup>3,7</sup>, but also due to increase in acidic amino acids like glutamic and aspartic acids. Hence, these studies indicate the presence of a general depressant in the body fluids, which could be in the nature of acidic amino acids in the aestivated snails.

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#### POST-OVULATORY CHANGES IN THE EGGS OF SOME INDIAN BATS

THE ovum has been shown to undergo shrinkage after ovulation and fertilization in several mammals (Please see Boyd and Hamilton, 1952; Blandau, 1961; for review of literature).

During the course of our studies on the reproduction and embryology of several species of Indian bats it was noticed that the egg undergoes a considerable shrinkage after ovulation and fertilization and retains the diminished size until the embryo

hatches out of the zona pellucida in all the species studied by us. Table I gives the diameter inclusive of the zona pellucida of the ovarian egg and of the early embryonic stages of development in the bats studied.

perhaps forms a barrier against the influx of material into the egg from the surrounding uterine lumen. However, after hatching out of the zona pellucida the embryo enlarges enormously before it establishes contact with the uterine wall. This

TABLE I  
Diameter of ovarian ova and early embryonic stages in  $\mu$

Name of species	Ovarian ovum	Unfertilized free egg	Fert. egg	Early cleavage	Late cleavage	Morula	Free uni. blastocyst
<i>Rousettus leschenaulti</i> ..	126	75	..	..	..	..	78
<i>Megaderma lyra lyra</i> ..	112	..	..	..	..	..	90
<i>Rhinolophus rouxi</i> ..	94	..	..	..	..	70	..
<i>Hipposideros speoris</i> ..	103	..	60	60	..	60	..
<i>Pipistrellus ceylonicus chrysotrix</i> ..	85	..	60	60	64	..	..
<i>Pipistrellus minimus minimus</i> ..	85	..	65	65	..	63	..
<i>Pipistrellus dormeri</i>	90	..	63	63	65	65	..

Two interesting facts come to light from Table I. (1) The diameter of the egg becomes markedly reduced after ovulation and fertilization, and if the volumes are compared the volume of the egg after ovulation and fertilization is less than half, and in some cases less than a quarter, of the volume of the ovum in the ripe Graafian follicle. (2) The embryo as a whole does not increase in size until it hatches out of the zona pellucida, but the cytoplasm of the egg is distributed to the daughter cells at successive cleavages and cell divisions. Hence, the cells of the embryo become progressively smaller as development proceeds. Evidently, the metabolic requirements of the embryo are provided by the stored material in the egg itself until the embryo hatches out of the zona, and perhaps there is not much influx of material into the embryo during this phase of the development of the mammalian embryo. Examination of the stained sections reveals that whereas the zona pellucida of the ovarian ovum in all the species studied here except *Rhinolophus rouxi* and *Pipistrellus dormeri* has cross striations and appears in surface views to be reticulate with non-staining meshes, the zona pellucida is homogeneous and shows no cross striations after ovulation. Apparently the zona pellucida undergoes some changes soon after ovulation and fertilization, and

enlargement is possible only by the intake of material by the embryo from the contents of the uterine lumen. Some evidence in support of this hypothesis is afforded by cases of mammals in which the blastocyst remains in an almost dormant condition in the oviducts during delayed implantation when there is no appreciable increase in the size of the blastocyst or in the number of cells. This would mean that whereas the nuclear material increases in quantity during successive divisions, the total amount of cytoplasm remains nearly constant as long as the zona pellucida is intact. It appears as though the cells of the embryo cannot undergo mitosis unless the embryo gets material from outside after the cells reach a certain minimum size.

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Nagpur, April 4, 1974. (MISS) GOPA RAJGOPAL.

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### AN UNDESCRIBED SPECIES OF *TREMATOSPHAERIA* FUECKEL FROM INDIA

AN interesting fungus growing saprophytically on the stems of *Randia dumetorum* Lam. (Family Rubiaceae) was collected by the writers in the forests of Castlerock (Mysore State) which on microscopic examination revealed the following characters:

Stroma black, innate-erumpent, carbonaceous; pseudothecia solitary, ostiolate; asci in basal layers, cylindrical to clavate, pedicellate, 8-spored, bitunicate; ascospores hyaline to dark-brown at maturity, ellipsoid to cylindrical, 4-celled, biseriate; paraphyses filiform, unbranched, hyaline. Based on these characters the fungus was identified as a species of *Trematosphaeria* Fuckel (Fam. Pleosporaceae). A critical perusal of literature further revealed that the genus *Trematosphaeria* has been represented amongst Indian fungi by only two species, viz., *Trematosphaeria jasmini* Chona *et al.*<sup>1</sup> and *T. abuensis* Panwar *et al.*<sup>2</sup>. The present collection was, therefore, critically compared with these two species as well as the other known and type species of *Trematosphaeria* and was found to be significantly distinct in respect of gross morphological characters, dimensions of pseudothecia, asci and ascospores. Hence, the same has been described here as a new species.

*Trematosphaeria microspora* Sp. Nov. (Fig. 1)

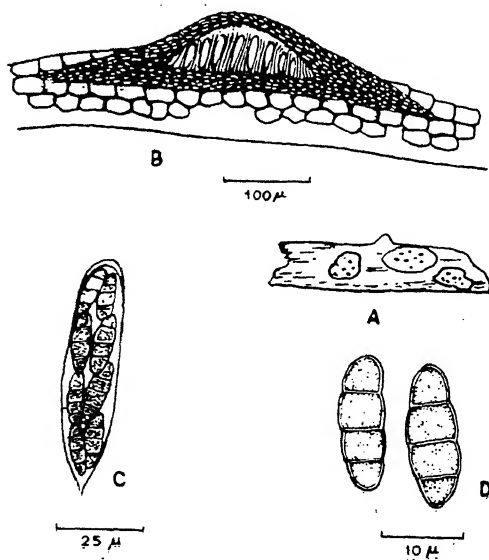


FIG. 1. *Trematosphaeria microspora*. A, Habit; B, Section through ascocarp (Pseudothecium); C, Ascus; D, Ascospores.

Fructificationes dispersa, separata, innata tandem erumpentia, nigra, carbonacea, magnit 340–510 μ

in diam.; asci in basilaria strata, cylindracea vel clavata, pedicellata, bitunicata, octospora, 40–72 × 10–16 μ; ascosporae biseriatae, hyalinae vel fusc-brunnae ad maturitas, ellipsoidae vel cylindraceae, 3-septatae, aliquando constrictae ad septum, apicibus rotundatis, magnit 16–20 × 6–8 μ; paraphyses filiformes, hyalinae, inramosae.

*Matrix*: In culmis emortuis *Randia dumetorum* Lam.'

Leg. D. N. Mhaskar, 21-10-1970 ad Castlerock-Anamod (Karnataka State). AMH\* No. 1902 (Holotypus).

The present collection markedly differed from the two Indian species of *Trematosphaeria* in colour and septation of the ascospores. Although it has some resemblance in this respect with the type species, viz., *Trematosphaeria pertusa* (Pers. ex Fr.) Fuckel, it differed from it in having significantly smaller asci and ascospores (asci 140 × 20 μ in the type as against 40–72 × 10–16 μ in the present specimen and ascospores 20–27 × 5–7 μ in the type as compared to 16–20 × 6–8 μ).

Grateful thanks are offered to Prof. M. N. Kamat for his keen interest and to Dr. J. A. von Arx, Director, C. B. S. Baarn (The Netherlands), for his help rendered in the identification of the fungus.

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D. N. MHASKAR,  
V. G. RAO.

October 11, 1973.

\* AMH: Ajrekar Mycological Herbarium at M.A.C.S., Poon-4 (India).

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### A NEW CROSSING TECHNIQUE IN CLUSTERBEAN [*CYAMOPSIS TETRAGONOLOBA* (L.) TAUB.]

A NEW crossing technique in clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] was developed during the last two years, i.e., *khurif*, 1972 and *khurif*, 1973. The technique has proved to be more efficient over the one already in practice. It not only leads to better effective pods but it also saves time in both emasculation as well as pollination. The proposed new technique is also simple and convenient to adopt.

In the usual technique in practice, the flowers likely to open after one or two days are selected and the stamens are removed with the pair of forceps by gently pushing the keels apart (Fig. 1a). Rest of the flowers and buds are removed (Fig. 1b). This eventually causes considerable damage to both

the selected bud as well as inflorescence. This ultimately results in the shading of flower buds (Fig. 1 c and d) after pollination process is over. This difficulty has often been considered as a serious limitation for the initiation of hybridization programme in clusterbean.

tion work. In order to avoid any damage, the upper buds are not removed until after 3 days of emasculation and care is taken to ensure that none of the upper buds open up during this period. Preferably, not more than two buds should be selected from the inflorescence at a time.

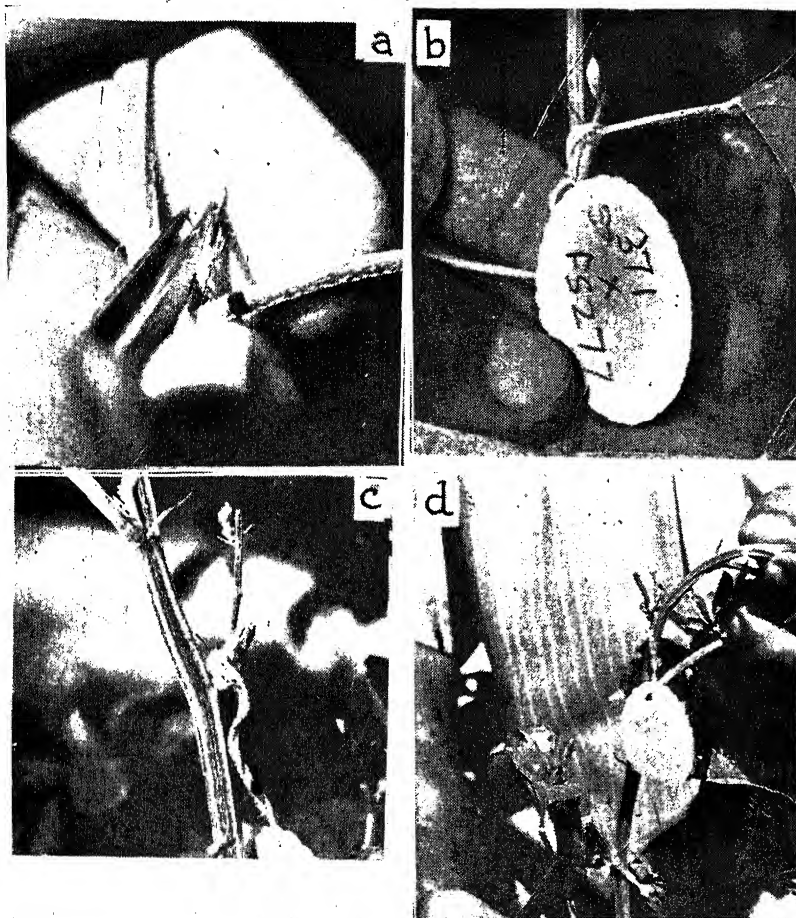


FIG. 1. Photographs showing usual crossing technique in clusterbean [*Cyamopsis tetragono-loba* (L.) Taub.].

The steps of the new technique are described below :

(1) The suitable buds are selected in the afternoon between 4 to 6 P.M. (Fig. 2 a). Care is to be taken while selecting the buds in such a way that the receptivity of the stigma remains at least for a day or two. Generally those buds are selected which can easily be pollinated the next morning.

(2) The remaining open flowers below the selected buds are removed (Fig. 2 b). Thus, the lowest bud is automatically the one used for emascu-

(3) The front sepal is removed through the help of forceps by gentle pulling (Fig. 2 c). This particularly helps in the removal of petals more conveniently.

(4) The petals are removed by gentle pulling in the forward direction with the help of forceps and necessary support to the bud is provided by the finger in such a way that only the petals along with almost all the stamens are removed together and no damage to the stigma or style is caused (Fig. 2 d). Soon after the removal of petals it is



also ensured that no anther remains intact (Fig. 2 e). Also it should be ensured at this stage that the anthers are undehiscent.

(5) In order to avoid the chances of cross-pollination, which are generally rare, the whole inflorescence is bagged using light butter paper bag.

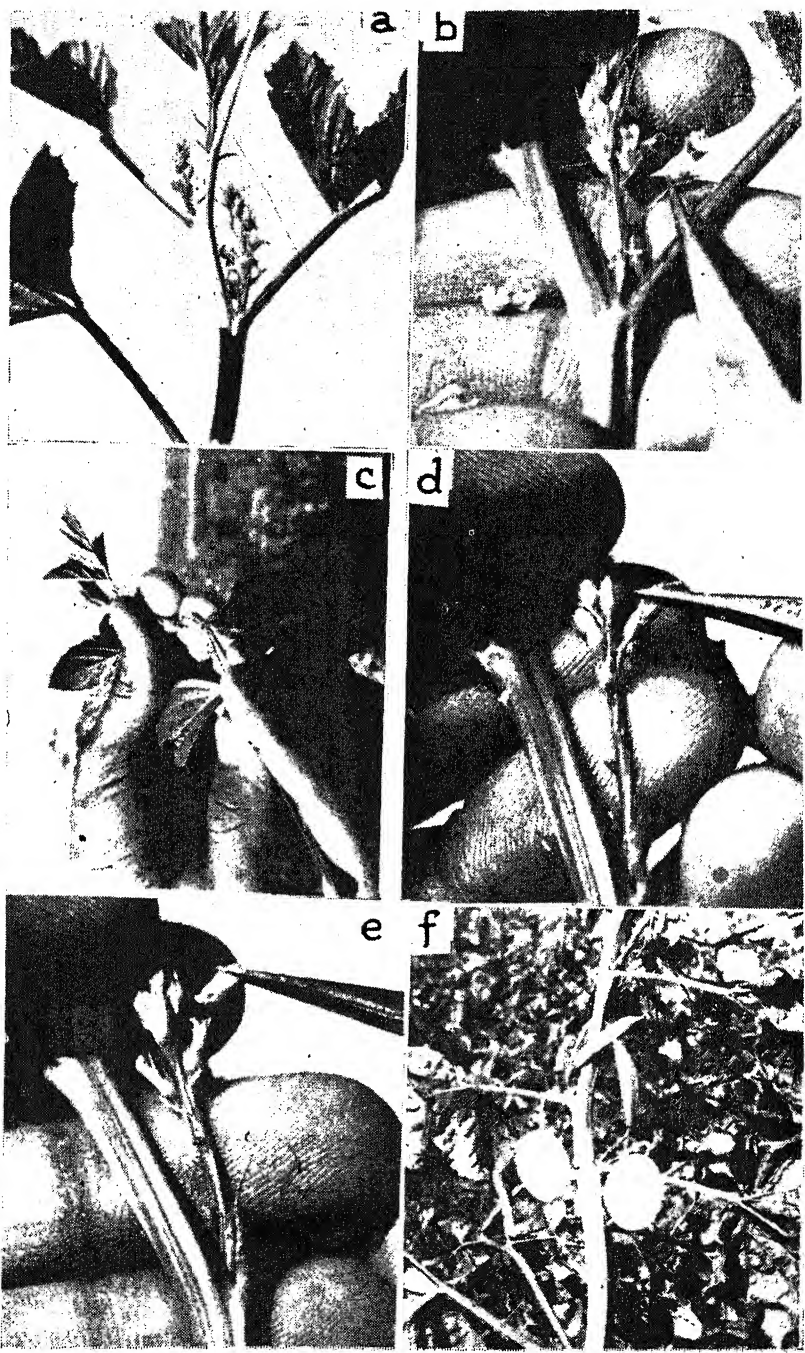


FIG. 2. Photographs showing the various steps of the new crossing technique proposed in clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.].

(6) The buds are pollinated in the morning, preferably between 8-9 A.M. In order to ensure perfect pollination, buds may be pollinated twice. The matured anthers, which generally burst on gentle touching, are directly brought to the contact of the stigma and the care is taken to not to damage the latter.

(7) Following pollination, proper labelling is done and the bud is bagged again. The bag should be removed immediately after the pod formation takes place. Generally, the pod formed after hybridization are relatively smaller in size with a few seeds (generally 2-3) per pod (Fig. 2). The shape and size by itself ensure easy identification of the crossed pods.

TABLE I

Data on relative efficiency of the proposed new crossing technique in guar over the usual technique in practice

			By usual techniques in practice	By proposed new techniques
Total buds used	Kharif, 1972	415	540	
	" 1973	376	1064	
Time required in emas- culation of 100 buds (minutes)	" 1972	151.0	50.50	
	" 1973	87.50	31.87	
Time required in pol- lination of 100 buds (minutes)	" 1972	105.24	39.60	
	" 1973	74.55	32.20	
Effective pods obtained	" 1972	12	23	
	" 1973	16	84	
Per cent effective pod setting	" 1972	2.40	6.0	
	" 1973	4.21	7.89	
Per cent efficiency over usual technique	Emasculation			
	Kharif, 1972	..	299.00	
	" 1973	..	274.55	
	Pollination			
	Kharif, 1972	..	265.75	
	" 1973	..	321.33	
	Pod setting			
	Kharif, 1972		250.00	
	" 1973		187.41	

It is evident from the data in Table I that the newly proposed technique is both simple as well as more efficient by almost 2-3 times over the usual technique in practice and thus, its use is advocated in order to undertake effective hybridization programme in clusterbean.

The authors wish to thank Dr. Kanwar Singh, Additional Director of Research-cum-Head, Department of Forage Research, for providing facilities.

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Haryana Agricultural Univ., R. S. PARODA.  
Hissar, January 15, 1974. K. R. SOLANKI.

# SEED SETTING IN *TABERNAEMONTANA* *DIVARICATA* BR. IN PLAINS

BESIDES the two well-known single and double-flower varieties of *Tabernaemontana divaricata* Br, which are diploid ( $2n=22$ ) and triploid ( $2n=33$ ), respectively, our studies revealed two more varieties<sup>1</sup>. One of these is diploid and differs from single-flower variety in the shape of corolla lobe, while the other is single-flower triploid variety. The latter resembles double-flower triploid variety in vegetative characters. In the plains of Uttar Pradesh all the varieties are totally sterile, and are being vegetatively propagated. However, seed setting does take place in the single-flower diploid variety in the hilly tracts of Himalayas. Our studies have revealed<sup>2</sup> that chromosome pairing in the single-flower diploid variety, under Lucknow conditions, is affected by seasonal variations. High pollen sterility, however, in this variety, was observed to be independent of chromosomal pairing.

We have been studying evolution in this species, at varietal level, besides artificial induction of polyploids and mutations, we have also been interested in restoring seed fertility so that we could trace various evolutionary steps.



FIGS. 1-2. Fig. 1. Immature fruits. Fig. 2. Splitting fruit showing 2 rows of seeds.

Induced mutations, as a result of gamma-ray exposure (Raghuvanshi and Singh, A. K.; Raghuvanshi and Singh, D. N.; unpublished), have so far not proved helpful in restoring fertility. During 1971, we obtained cuttings from a plant growing at Kathgodam (foot hills of Himalayas). The rooted cuttings have been flowering for quite some time and, during 1974, we were somewhat surprised

to find fruit formation on one of the plants. Each flower has two carpels which, ultimately, form a follicle. The fruits in the beginning were green and appeared leaf-like but, gradually, they matured. This is a very slow process and takes about four months. When the fruits ripe, they split open exposing the red interior. The seeds were slightly elongated with a hard testa of red colour. Each fruit has about 12-17 seeds. The success in obtaining seed-setting has opened up new possibilities in breeding this ornamental.

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Botany Department,  
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Lucknow, India, February 11, 1974.

S. S. RAGHUVANSHI  
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#### CYTOLOGICAL STUDIES IN *BLASTINIA GRACINI* CONG. OF *CUCURBITACEAE*

*Blastinia gracini* grows wildly as climber in North India<sup>2</sup>. The collection for the present investigation was made from Bichpuri, Agra. The genus is monotypic<sup>3</sup> and is characterised by simple tendril, five deeply lobed cordate leaves, straplike bracts, male flowers in raceme on inconspicuous axile peduncle, calyx tube small, corolla five, stamens three, female flowers solitary axillary, fruit small globose deep red on ripening and seeds are ovoid boat-shaped.

For cytological studies young flower buds were fixed in (1 : 3) acetic-alcohol. Anthers were squashed in 1.5% acetocarmine. Temporary slides were used for photography and detail study.

Analysis of large number of pollen mother cells at diplotene and diakinesis shows regular twelve bivalent formations along with a prominent nucleolus attached to one or two bivalents (Fig. 1).



FIG. 1. A pollen mother cell at diplotene showing 12II,  $\times 2,400$ .

At metaphase I, 6-8 ring bivalents and 4-6 rod bivalents were observed. Further stages of meiosis were found regular.

The base number for this genus is not known. A large number of genera in the family *Cucurbitaceae* have twelve as base number<sup>1</sup> and the present investigation suggests twelve as the base number for this genus too. Furthermore, the present study also indicates a diploid nature of the species.

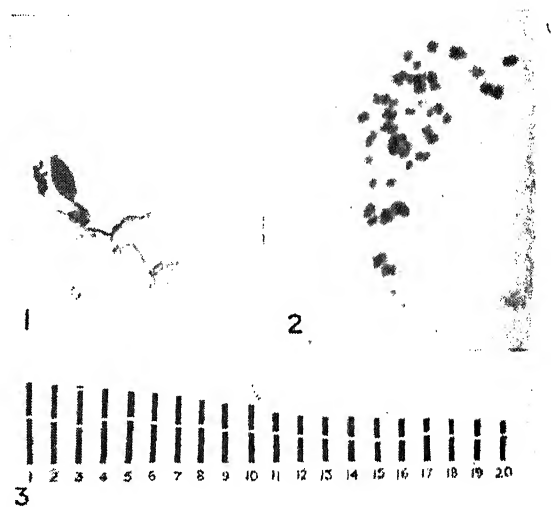
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A. K. SINGH.

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#### KARYOMORPHOLOGICAL STUDIES IN *BULBOPHYLLUM NEILGHERRENSE* Wt.

*Bulbophyllum neilgherrense* Wt. is an epiphytic orchid belonging to the subtribe *Dendrobium* Benth. of *Orchidaceae*<sup>3</sup>. The plant (Fig. 1) is



FIGS. 1-3. Fig. 1. Photograph of the plant. Fig. 2. Photomicrograph of the metaphase plate showing  $2n = 40$ . Fig. 3. Idiogram,  $\times 3,330$ .

locally known as "Purusha Ratna" and is used as tonic in Ayurvedic system. It is distributed throughout the Western Ghats and other parts of South India. Although there are reports of chromosome numbers of many species in the

genus<sup>1,2,4-6</sup>, detailed karyomorphological study is meagre. The present study, therefore, deals with the chromosome number and the detailed karyotype of this species.

The material for the present study was collected from Sirsi, North Kanara District, and is grown in the Orchidarium of the Botany Department, Karnataka University, Dharwar.

The chromosome morphology was studied following Tjio and Levan's technique<sup>7</sup>.

The diploid chromosome number of this plant is found to be  $2n = 40$  (Fig. 2) and is the first time report. The idiogram shows that the chromosomes fall in a series of close gradations (Fig. 3). The study of karyotype reveals that 17th and 18th pairs of chromosomes are with submedian constrictions and all the rest of the chromosomes are with either median or near median constrictions. The third pair is distinguishable because of its secondary constrictions on the short arm. The length of the chromosomes varies from 1.50 microns to 3.23 microns.

Hitherto, chromosome numbers of about 29 species have been reported for the genus, *Bulbophyllum*. Haploid chromosome number ( $n$ ) of 8 species has been reported as 19 and of 4 species as 20<sup>1-4</sup>. Diploid chromosome number ( $2n$ ) of three species has been reported as 38 and of 10 species as 40<sup>2,4,5</sup>. However, in the present investigation, the diploid chromosome number for this species is found to be  $2n = 40$ .

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### REMARKABLE SIMILARITIES IN POLLEN DYNAMICS

ALL adaptive devices of pollen, leading from the male reproductive unit to the female counterpart, in order to effect fertilization, come under the purview of pollen dynamics. The functional purpose of pollen with adaptive devices in its structure and function coupled with those of the vegetative and reproductive organs is met with in a variety of forms of different species<sup>1-4</sup>. For instance, in

structural organization, the pollen are similar to spores, fruits, seeds and sometimes entire organisms. Monads are analogous to unicellular spores and many diatoms; dyads in Podostemaceae and Scheuchzeriaceae to 2-celled ascospores of *Nectria*; tetrads in Droseraceae and Juncaceae to spore tetrads of *Sphaerocarpos*; polyads in Mimosaceae and Orchidaceae to spore massulae of *Azolla* and *Salvinia*; tetrads arranged in thread-like chains in *Halophila* to unbranched filamentous blue green alga like *Oscillatoria*; winged pollen of *Pinus* to winged fruits of *Acer*; pollen of grasses provided with air spaces in their wall to seed coat of *Limnanthemum*<sup>2,5-10</sup>. In function, the budding during pollen development of *Petunia* is comparable to yeast fungi; pseudomonad development in Cyperaceae and tribe Styphelieae of Epacridaceae where only one microspore in a tetrad develops to monosporic female gametophyte development in angiosperms and also the megaspore development in *Selaginella rupestris*<sup>6,11-13</sup>.

The random occurrence of similarities in structures meant for different functional purposes such as pollen for fertilization, spores, fruits and seeds for propagation, dissemination and perpetuation respectively, in species of remote taxonomic groups of different habits and habitats such as the torrential thalliod Podostemaceae, herbaceous marshy Scheuchzeriaceae, microscopic parasitic fungi like *Nectria*, filamentous aquatic *Oscillatoria* and the wonderfully ornamented diatoms indicate that each similarity in that particular structure or species may bear its own rate of adaptive significance which in a way lessens the severe competition in those adapted to the same ecological niche. In this context, further critical study in similarities in addition to other features such as geometric design (size, shape and pattern of construction), number of representatives, contents of the pollen such as the number of nuclei and cytoplasmic inclusions is essential to discover the exact mechanisms correspondingly adapted to the mode of sexual reproduction<sup>8,14-15</sup>. It appears that during the course of evolution, of pollen (also spores), organization into any pattern of single or compound structure happens to be the basic feature on or after which further variation might have evolved resulting in diversified types, thus recalling the same pattern observed in the evolution of unicellular and multicellular plants in particular and organisms in general<sup>15-16</sup>.

In pollen producing plants, the gymnosperms have lesser number of species representatives with limited variation in pollen having only monads as compared to angiosperms where relatively more species are present with a varied array of pollen types

ranging from monads, dyads, tetrads to polyads. Even the pattern of sculpturing is more in angiosperms. Considering from these points, the search for causes for such differential rates of evolution and existence, the influence of climatic effects on the present-day dominant pollen and spore producing plants should be there to understand their timely adaptation and future in a better manner.

We thank Dr. K. Subramanyam for scrutinising the manuscript.

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#### OCCURRENCE OF HYPOGYNY IN CUCURBITACEAE

CUCURBITACEAE, the gourd family, is distinguished by the herbaceous prostrate or scandent habit, morphologically controversial tendrils, unisexual flowers, androecium with unusual modifications, and above all these the most prominent one is the inferior ovary.

While studying the pollination ecology of cucurbitaceous plants, it was observed that muskmelon (*Cucumis melo* Linn.), unusually produced bisexual hypogynous flowers along with the normal epigynous unisexual/bisexual flowers.

Muskmelons are generally cultivated during summer season only. But for the sake of pollination study this crop was maintained in all the seasons of the year. During summer and rainy seasons three types of flowers were noticed to appear on the same plant. Presence of separate pistillate and staminate flowers represented the usual monoecious, unisexual character of the family. Whereas the third type was represented by bisexual flowers, a feature not very strange among most of the cucurbits. Epigyny was the constant feature of both the pistillate as well as the bisexual summer flowers (Figs. 1, 2, 3).

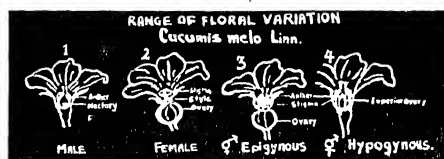


FIG. 1

In these epigynous flowers the inferior ovaries were spherical and ovoid, having a diameter of about one cm. The styles were short and were capped by three obtuse stigmas.

During the course of winter season a fourth type of flower added itself to the list of normal summer types. The latter kind was bisexual hypogynous (Fig. 4). Throughout the winter all the four types of flowers regularly made their appearance. On the basis of the ratio worked out, it was established that among every 12 normal flowers, there was 1 abnormal hypogynous bisexual flower. Outward view of these flowers gave an impression of perfect similarity with the normal staminate flowers. But, in fact a minute superior ovary was present between the staminal column in each of such flowers. Apparently such ovaries could not be located because anthers at the mouth of the calyx tube overshadowed them.

The gynoecium in these abnormal winter flowers was found to differ completely in shape from that of epigynous flowers. The superior ovaries in the hypogynous flowers were cylindrical with a diameter of two mm, and a length of four mm. Styles were completely reduced and three hyaline radiating glandular notches represented the stigmas. The arrangement of ovules in these minute cylindrical superior ovaries was practically the same as that in the normal inferior ovaries.

Occurrence of hypogyny in this group appears to form a feature of a special significance as nowhere in the available literature could there be found any mention of hypogyny in Cucurbitaceae. Perhaps attempts could be made to analyse if this feature could safely be attributed to the variations

caused in the differentiating cells of the decapitated portion of the seedling. The results of this experiment are in accordance with the findings of other workers. For details, with references, see the paper of detail, with references, given above.

I have to thank the Director, Dr. S. D. Saxena for his interest in the work and for the provision of the facilities. Dr. S. Bhattacharya for his interest in the running of the laboratory and Dr. S. S. Chakravarti for his interest in the work.

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# VERNALIZATION OF EPIDERMAL CELLS IN LINSEED

In young seedlings of *Linum catharticum* L., square, I have observed that the apical meristem may become inactive and the plant may tend to differentiate into a vegetative state. Chakravarti recorded that the apical meristem of a vernalized seedling of *Linum* is inactive in the parts on the sides of the apical meristem, leaving epidermal cells on the sides of the apical meristem, leaving the vernalization stimulus. My present experiments, decapitation and the effect of the plant, were on day-old seedlings of *Linum* to see whether the

portion, in paper cups. Ten days after germination, the seedlings were decapitated and kept for three weeks in a refrigerator maintained at about 5°C. After the treatment was over, they were transplanted in flower pots along with similar decapitated normal seedlings. On this day, seeds vernalized for the same period were also sown along with control ones. Observations consisted of recording date of anthesis and counting of number of leaves developed prior to it in all the four treatments. To determine whether the cells of the epidermis divided during chilling about a time and portion taken from different parts of the hypocotyl were stained with aqueous safranin, mounted in glycerine and studied under the microscope.

Data presented in Table I reveal that chilling caused a significantly lower time of flowering accompanied by a reduction in the number of leaves in both intact and decapitated plants. As no directional traces have been observed in the epidermal peels examined, it may be concluded that perception of vernalization stimulus by the epidermal cells of *Linum* is independent of the apical meristem. Better response seen in intact plants in the present and the previous work might be due to shoots resulting in them from several cells of the apical meristem subjected to low temperature in comparison to the single one of the epidermis.

TABLE I

Effect of vernalization on the time and duration of flowering of *Linum catharticum* on the time taken for anthesis and number of leaves developed prior to it. Number of plants are given within brackets.

Treatment	Age (days)	Earliness	Leaf No.	Flowering
Control intact	22.1 (15)		90.3 (15)	—
Vernalized "	22.5 (15)	19.9 ± 0.6	45.5 (15)	33.8 ± 6.1
Control decap	22.6 (15)		43.8 (25)	—
Vernalized "	22.1 (15)	9.5 ± 1.5	43.2 (25)	10.1 ± 2.5

observed effect was due to the diffusion of the product of chilling from apical meristem to cells of the epidermis. Though in the existing literature there is no evidence to show that the product of vernalization could be transferred from cell to cell by any process other than that of auxin<sup>1,2</sup>, to be on the safe side, it was thought proper to test whether epidermal cells of *Linum* could perceive cold vernalization in the absence of the apical meristem.

For the experiments, seeds of strain Mahoba were sown in a mixture of soil and sand in equal pro-

Dept. of Botany, S. C. CHAKRAVARTI,  
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Bhopal, March 16, 1974

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 REVIEWS AND NOTICES OF BOOKS
 

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**Immunology Series (Vol. 1) Mechanisms in Allergy: Reagin Mediated Hypersensitivity.** Edited by Lawrence Goodfriend, Aloc H. Sohon, and Robert P. Orange. (Marcel Dekker, Inc., 95, Madison Avenue, New York, N.Y. 10016), 1973. Pp. xviii + 573. Price \$ 26.50.

This first volume in the *Immunology series* presents proceedings of the International Symposium on 'Control mechanisms in Reagin-Mediated Hypersensitivity' held at Montreal in 1972.

A critical appraisal of the new information and perspectives in this field of immunology are special features of this volume.

The role of allergen, particularly in parasitic infections, the interaction of thymus and bone marrow-derived lymphocytes, *in vitro* correlates of human atopy and the genetic basis of reagin production were the topics of discussion in the first two sessions.

T cell participation in both induction and termination of  $I_H E$  antibody formation and the control of immune responsiveness by Ir genes linked to major histo-compatibility locus were the highlights of these discussions.

The third and fourth sessions dealt with interaction of reagins with homologous and heterologous target cell receptors resulting in the ultimate release of vasoactive constituents from mast cells and basophils; the use of polypeptide histamine liberators to predict the amino acid sequence of the fragment of Fc region of  $I_H E$  antibody responsible for the target cell activation; the presence of two distinct homocytotropic antibodies in man and the role of haptens in both inducing and inhibiting allergic reactions.

The last two sessions focus attention on biochemical mechanisms underlying the  $I_H E$  mediated release of chemical mediators, methods of pharmacological modulation, the nature of the potential mediators and the results of their interaction and the clinical application of these new advances in allergy research.

M. SIRS.

**Principles of Reaction Mechanism in Organic Chemistry.** By V. S. Parmar and H. M. Chawla. (Sultan Chand and Sons, Publishers, 4792/23, Daryaganj, Delhi-110006), 1973. Pp. xii + 374. Price : Students Edn. Rs. 10.00 ; Library Edn. Rs. 15.00.

The authors have intended this book to be useful to teachers, undergraduate and post-graduate students. Perhaps, it has partly served the needs of the above. It is a useful first book for a beginner interested in knowing about organic reaction mechanisms. For post-graduate students and teachers, there is nothing special in this book that is not available in standard text-books like Fieser or Monison and Boyd, etc. The book would have been more useful to teachers and post-graduate students if more bibliography were given under each chapter for further reading rather than list a bunch of books at the end of the book. Further the authors do not give more recent literature on the topics.

The book has been divided into two parts. In Part I, the authors have introduced the modern concepts to understand the actual reaction mechanisms discussed in Part II.

For a book of the size of 374 pages, the index of 2 pages is inadequate.

T. R. KASTURI.

#### Books Received

**Interpretation of Mass Spectra (2nd Edition).** By F. W. McLafferty, Addison-Wesley/W. A. Benjamin, Inc., Advanced Book Program, Reading, Massachusetts 01867), 1973. Pp. xix + 278. Price: Cloth binding \$ 15.00, Paper \$ 7.50.

**Mossbauer Effect and Its Applications.** By V. G. Bhide. (Tata McGraw-Hill Pub. Co., C. 98-A, South Extension, Part II, New Delhi 110049). 1973. Pp. viii + 491. Price Rs. 75.00.

**Epoxy Resins Chemistry and Technology.** Edited by Clayton A. May Yoshio Tanaka. (Marcel Dekker, Inc., 95, Madison Avenue, New York, N.Y. 10016), 1973. Pp. xii + 801. Price \$ 59.50.

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# KINETICS AND MECHANISM OF HYDROLYSIS OF MONO-*p*-IODO BENZYL PHOSPHATE (MONO AMMONIUM SALT)

M. M. MHALA AND A. V. KILLEDAR\*

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## ABSTRACT

Hydrolysis of mono-*p*-iodobenzyl phosphate has been investigated in buffer solutions ranging from pH 1.00 to 8.04 at 80° C. Both neutral and mononegative species have been discovered as reactive species whose zones are demarkated in pH-log rate profile. Unlike monoaryl phosphates, mono-*p*-iodo benzyl phosphate does not show distinct maximum at ~ pH 4. Reactivity of the ester in buffer solutions decreases considerably than in acidic medium. Theoretical rates estimated from presumed pK values have been found to be in close agreement with the experimental rates. pK values have also been used to isolate rates of different species by determining their fractions. Arrhenius parameters, isokinetic relationship and comparative kinetic rate data have been used to propose the probable mechanism of the reaction.

THE importance of phosphoric acid derivatives and the role of phosphate linkages in Biochemistry are well understood. Consistently the kinetics of reactions of simple organic phosphates provides an insight into more complicated reactions occurring during their metabolism. Monoaryl phosphates hydrolyse rapidly at about pH 4 involving P-O fission of monoanions. Dianions of dinitrophenyl and *o*-carboxyl phenyl phosphates have been shown to be more reactive than their monoanions<sup>1</sup>. Very little is known about the behaviour of benzyl phosphates<sup>2</sup>. There is almost no record about the synthesis and kinetic study of hydrolysis of mono-*p*-iodo benzyl phosphate. Due to structural differences mono-*p*-iodo benzyl phosphate is expected to exhibit entirely different reaction paths than those envisaged for mono alkyl and aryl phosphates.

## MATERIALS AND METHODS

Mono-*p*-iodo benzyl phosphate (mono ammonium salt) was prepared<sup>3,4</sup> by the step-wise degradation of Tri-*p*-iodo benzyl ester (Found : P = 9.28%, N = 4.41%, C = 24.82% and H = 3.28%, C<sub>7</sub>H<sub>11</sub>PO<sub>4</sub>NI requires : 9.36%, 4.23%, 25.40% and 3.35% respectively). The hydrolysis of mono-*p*-iodo benzyl phosphate (mono ammonium salt) (0.0005 M) was followed by colorimetric estimation of inorganic phosphate by the method of Allens<sup>5</sup>. Interpolated values of buffers at 80° determined from the work of Stenc<sup>6</sup> were used. All the chemicals used were of B.D.H. (A.R.) quality.

## RESULTS AND DISCUSSION

**Hydrolysis via neutral species.**—The rate of hydrolysis of mono-*p*-iodo benzyl phosphate in the region pH 1.0 to 2.0 (Fig. 1) is represented by

$$k_t = k_N \cdot \frac{N}{N+M} + k_{M_0} \cdot \frac{M}{N+M} \quad (1)$$

where  $k_t$ ,  $k_{N_0}$  and  $k_{M_0}$  are total rate, specific neutral rate and specific mononegative rate respectively;  $N/N+M$  and  $M/N+M$  are the fractions of neutral and mononegative species respectively. The calculated  $k_{N_0}$  value is  $6.25 \times 10^{-4} \text{ min}^{-1}$ , which is different from the mononegative rate ( $k_{M_0} = 1.33 \times 10^{-4} \text{ min}^{-1}$ ) and the specific acid catalysed rate ( $k_{H_0} = 39.8 \times 10^{-4} \text{ min}^{-1}$ ).

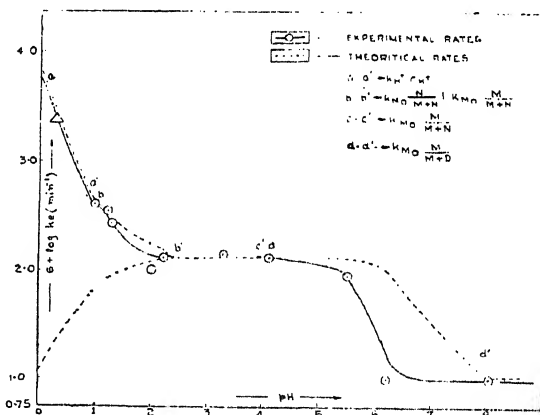


FIG. 1. pH log rate profile for the hydrolysis of mono-*p*-iodo benzyl phosphate at 80° C (Δ rate at 0.5 M HCl acid).

Table I shows the agreement between the estimated and observed rates. The slope of the linear plot in this region is almost unity indicating that monoprotonated form of the bulk mononegative species (Neutral) are reactive<sup>7</sup>.

Effects of factors such as temperature solvent, etc., on the rate of reaction via neutral species could not be determined due to the concomitant hydrolysis of mononegative species in this region and due to negligible contribution of this reaction in acid region, because of the masking of this reaction by acid catalysis<sup>8</sup>. The neutral species

\* Lecturer in Chemistry, Government College, Dhulpur (Raj.).

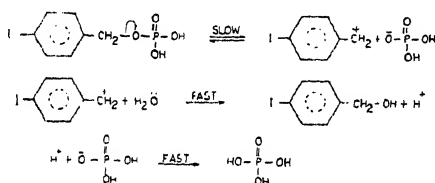
TABLE I

Calculated and observed rates for the hydrolysis of mono-*p*-iodo benzyl phosphate at 80° C (From  $pK_1$ )

pH	M N+M	$10^4 k_M$ (min. <sup>-1</sup> )	N N+M	$10^4 k_N$ (min. <sup>-1</sup> )	6 + log $k_e$ Calcd.	Obsd.
1.00	0.500	0.67	0.500	3.12	2.58	2.59
1.23	0.629	0.84	0.371	2.32	2.50	2.54
1.30	0.666	0.89	0.334	2.09	2.47	2.42
2.00	0.909	1.21	0.091	0.56	2.25	2.00
2.20	0.941	1.26	0.059	0.37	2.21	2.11
3.30	0.961	1.28	0.039	0.24	2.18	2.15
4.00	0.999	1.33	0.001	..	2.12	2.11

of the ester is more likely to be cleaved at C-O linkage rather than P-O bond, as the *p*-iodo benzyl cation formed subsequently as the reaction intermediate would be greatly stabilised by the +M (electron releasing) effect of the iodosubstituent. This view is substantiated from the comparative kinetic data of other related monoesters (Table II). Mechanism of the hydrolysis of monoester *via* neutral species may therefore be represented as shown in Chart 1.

(A) UNIMOLECULAR HYDROLYSIS WITH C-O FISSION -



(B) BIMOLECULAR HYDROLYSIS WITH C-O FISSION -

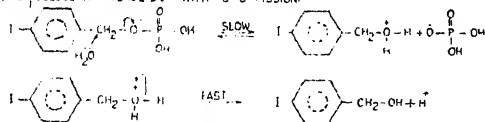


CHART 1. Probable reaction paths for the hydrolysis of mono-*p*-iodo benzyl phosphate *via* neutral species.

TABLE II

Comparative rate data for the hydrolysis of monophosphate esters *via* neutral species  
Solvent—Water

Phosphate ester	$k_{30} \times 10^4$ (min. <sup>-1</sup> )	Temp. °C	$pK_1$ value	Bond Fission
Methyl <sup>9</sup>	.. 0.30	100.1	1.6	C-O
Benzyl <sup>2</sup>	.. 0.60	75.6	..	C-O*
<i>p</i> -Iodo benzyl	6.25	80.0	1.00	C-O*
Allyl <sup>10</sup>	.. 6.20	80.0	1.00	C-O*

\* Fission presumed.

*Hydrolysis via mononegative species.*—The ester differs from aryl phosphates in not showing a distinct maximum at  $\sim$  pH 4. The dissociation constant value ( $pK_1=1.00$ ) of the ester has been calculated by considering the following equilibrium and presuming the hydrolysis of monoester to be exclusively *via* monoanion at pH 4 :

Neutral species  $\rightleftharpoons$  Mononegative species +  $H^+$

$$\frac{K_1}{K_1 + H^+} = \frac{M}{M + N} \quad (2)$$

Theoretical rates *via* neutral and mononegative species are estimated as follows :

$$k_N = k_{N1} \cdot \frac{N}{N + M} \quad k_M = k_{M0} \cdot \frac{M}{N + M} \quad (3)$$

Table I summarises the estimated and observed rates. Unlike aryl phosphates, the monoanion of this ester is considerably less reactive than its conjugate acid species ( $k_{M0} = 1.33 \times 10^{-4}$  min.<sup>-1</sup> while  $k_{N0} = 39.8 \times 10^{-4}$  min.<sup>-1</sup>).

In the region pH 4 to 8, the presumption of dissociation of monoanion into dianion and a proton ( $pK_2=7.00$ ) permits estimation of the rates of mono and dinegative species. Results (Table III) show that the reaction is exclusively governed by mononegative species.

TABLE III

Calculated and observed rates for the hydrolysis of mono-*p*-iodo benzyl phosphate (From  $pK_1$ )

pH	M M+D	D M+D	$10^4 k_M$ (min. <sup>-1</sup> )	6 + log $k_e$ (Calcd.)	Obsd.
4.12	0.999	0.001	1.33	2.12	2.11
5.51	0.969	0.031	1.29	2.11	1.98
6.20	0.863	0.137	1.15	2.06	1.04
8.04	0.084	0.916	0.11	1.04	1.04

Arrhenius parameters (Fig. 2) for the hydrolysis at pH 4.12 have been found to be  $\Delta S^\ddagger = -17.37$

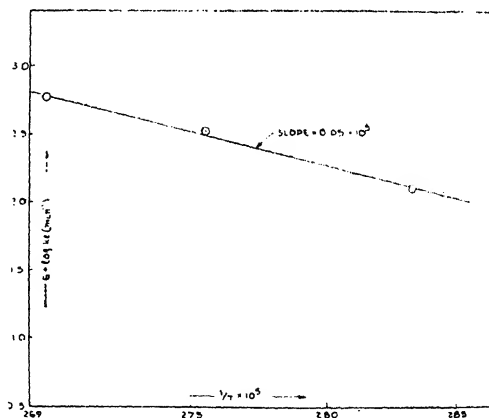


FIG. 2. Arrhenius plot for the hydrolysis of mono-*p*-iodo benzyl phosphate at 4.12 pH.

e.u.,  $E = 22.89$  K.cal/mole and frequency factor  $= 3.20 \times 10^{10}$  sec.<sup>-1</sup>. These results are consistent with bimolecular<sup>11</sup> nature of the reaction. The monoanions of both alkyl and aryl phosphates have been shown to hydrolyse exclusively via phosphorus-oxygen bond fission<sup>12,13</sup>. Isokinetic relationship (Fig. 3) shows a linear plot suggesting

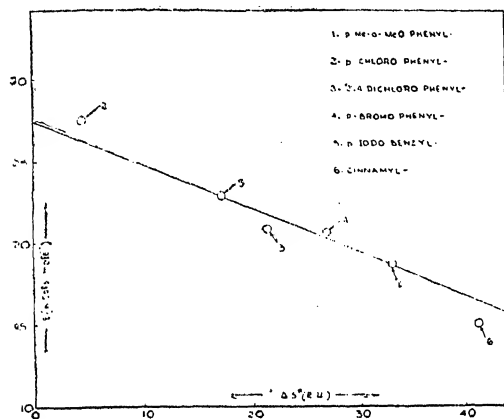


FIG. 3. Isokinetic relationship for the hydrolysis of phosphate monoesters via mononegative species.

similarity of mechanism for the hydrolysis of this ester and other related phosphates. Based on the results gathered, the probable reaction paths may, therefore, be formulated as shown in Chart 2.

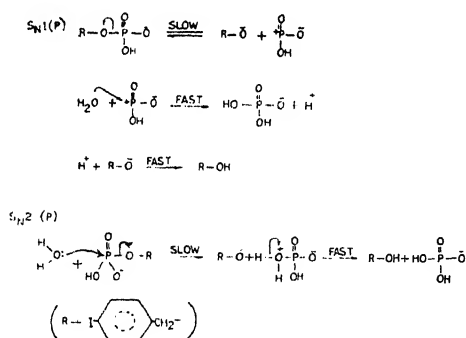
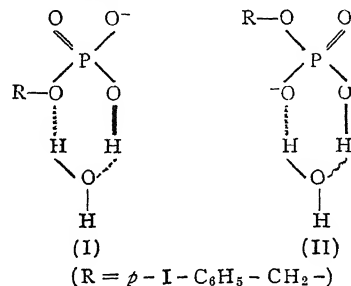


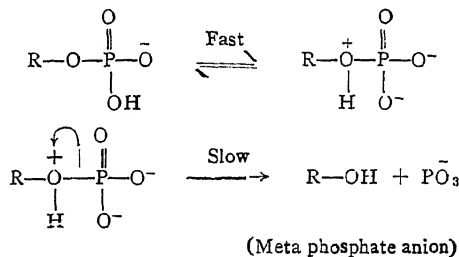
CHART 2. Mechanism of the hydrolysis of mono-*p*-iodo benzyl phosphate via monoanion.

The reaction paths via mononegative species may also be represented by rapid formation of the hydrogen bonded complexes (I) and (II) with water, which readily decompose with Phosphorus-Oxygen bond fission. (II) is preferred over (I) since the rate of hydrolysis increases with electron

attracting power of the substituent<sup>13</sup>, which will not favour hydrogen bonding as shown in (I).



As the rate of hydrolysis increases by a change-over from water to deuterium oxide as a solvent, ( $k_{\text{D}_2\text{O}}/k_{\text{H}_2\text{O}} = 2.36$ ), following mechanism may also be suggested:



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## LEVELS OF LYSOSOMAL HYDROLASES DURING GROWTH OF YOSHIDA ASCITES SARCOMA

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## ABSTRACT

The profiles of six lysosomal acid hydrolases, *viz.*, acid phosphatase, acid ribonuclease,  $\beta$ -glucuronidase, arylsulfatases A and B, and cathepsin D were studied during the progression of the fast growing Yoshida ascites sarcoma. The initial high activities of the enzymes declined with tumour age during the period of active cell multiplication. However, they showed marked increase during the terminal phase when there was increased cell loss due to cell destruction and autolysis. An increase in the proportion of free activity to bound levels was also found during the terminal phase. This shift towards the "free" state may reflect intra-cellular release of the enzymes in the older tumour cells.

## INTRODUCTION

**L**YSOSOMAL enzyme activities are known to increase during processes involving tissue degradation, as in regression of tumours, either spontaneous or induced by irradiation<sup>1</sup> or hormonal deprivation<sup>2</sup> or treatment with drugs<sup>3</sup>. Studies on solid tumours indicated that hydrolase levels remain low in regions of active growth, but are high in necrotic or senescent areas<sup>4</sup>. There is scanty information about the relationship of lysosomal enzyme levels to growth of ascites tumours where there is no demarcation between growing and senescent regions. However, in an ascites tumour like Yoshida ascites sarcoma (YAS), after rapid proliferation during the first few days, involution as well as loss of a certain population of cells from the proliferating fraction occur. Therefore the involvement of lysosomes in this process was studied and quantitated and the results are presented below.

## MATERIALS AND METHODS

**Tumour.**—Yoshida ascites sarcoma was maintained in Wistar A/lisc rats by serial intraperitoneal transplantation of 30–40 million cells every 4th day. On various days after transplantation, the tumour cells were collected in 10-fold excess of physiological saline from the peritoneal cavity after sacrificing the animal by cervical dislocation. The washings of the peritoneal cavity were pooled with the original sample and the YAS cells were counted in a haemocytometer.

**Preparation of cell-free extract.**—All operations were carried out at 4° C unless stated otherwise. The ascites cells in saline suspension were freed from contaminating erythrocytes by centrifuging and washing with hypotonic saline and finally centrifuged at 10,000 g to give a hard cell pellet. The cells were suspended in 0.25 M sucrose (10% w/v suspension) and sonicated for 5'. The sonicate was then centrifuged at 10,000 g for 20'. The supernatant (cell-free extract) was used immediately to assay the total enzyme activities.

**Preparation of subcellular fractions.**—The hard cell pellet obtained as above was ground in an all-glass mortar for 10'; phase contrast microscopy at this stage revealed more than 95% cell breakage with little damage to the particles. Differential centrifugation was used to separate nuclei and cell debris (1,200 g pellet), particulate or lysosomal fraction (15,000 g pellet), microsomes (105,000 g pellet) and cytosol (105,000 g supernatant). The lysosomal fraction was suspended in 0.25 M sucrose.

**Assay of enzymes.**—Acid phosphatase<sup>5</sup> and  $\beta$ -glucuronidase<sup>6</sup> were assayed by the hydrolysis of their respective *p*-nitrophenyl esters under optimal conditions of temperature and pH. Arylsulfatases A<sup>7</sup> and B<sup>8</sup> were assayed with *p*-nitrocatechol sulfate as substrate and cathepsin D<sup>9</sup> by following the hydrolysis of acid-denatured bovine haemoglobin at pH 4.0. Acid ribonuclease<sup>10</sup> was assayed by the release of acid soluble nucleotides from purified yeast RNA. In the case of the lysosomal fraction, the enzymes were assayed in the presence of 0.1% Triton X-100.

All activities were expressed as  $\mu$  moles of product formed/hr/mg protein except acid ribonuclease, where the activity was expressed as units/mg protein. One unit of activity was that amount of enzyme which caused an OD change of 1.0 at 260 nm at 37° C in 30 min.

## RESULTS AND DISCUSSION

The growth curve of YAS is shown in Fig. 1. There was rapid growth during the first three days. The cell population reached a maximum on the fourth day and thereafter declined. The mean survival period of the tumour bearing rats was six days. The mean growth rate showed continuous deceleration even during the period of rapid growth, and fitted well with a Gompertzian function<sup>11</sup>. The retardation of growth in the later stages may reflect a situation similar to that in Ehrlich ascites tumour<sup>11</sup> where a growth retardation results from

a prolongation of cell cycle time and a moderate decline in the proliferating fraction with a slow increase in the rate of cell loss.

plasm, karyolysis and chromosomal breakages and autolysis, leaving a population of dormant "stem-line cells" which proliferate when transplanted to

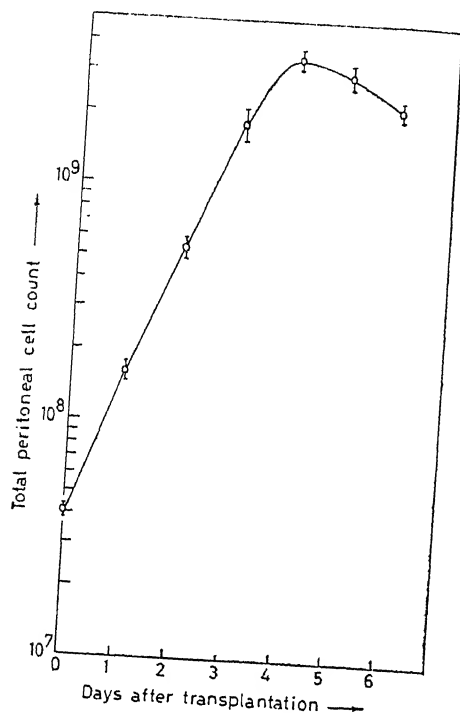


FIG. 1. Growth curve of Yoshida ascites sarcoma in Wistar A/lsc rats. Each point represents mean  $\pm$  S.E. of 5 experiments.

Figures 2 and 3 show the profiles of the six lysosomal hydrolases during tumour progression. All of them followed, in general, the same pattern of a decline in activity during the period of rapid growth. Following transplantation, except the stem-line cells, most of the mature YAS cells which form the major population in the inoculum undergo degeneration. This could account for the high activities of the lysosomal enzymes on the first day, since they are involved in tissue degradation. Thereafter the stem-line cells start rapid multiplication until the fourth day, when the proportion of cells undergoing degeneration and lysis again increases. Associated with this process there is a rise in lysosomal activity also.

The data in Table I indicate that the percentage of free activity was the highest when the tumour was in the senescent or regressing stage. This would indicate an internal labilization of the lysosomal membrane coupled with elevated activities of the lysosomal enzymes. This would explain the cytological changes reported<sup>12</sup>. Most of the cells of old YAS tumours undergo vacuolation of

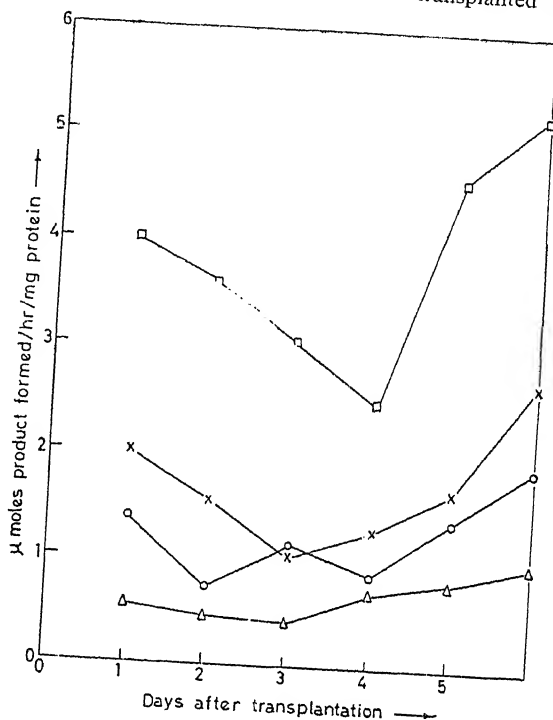


FIG. 2. Levels of four acid hydrolases (free plus bound) in YAS during tumour progression. Data are mean of three experiments.

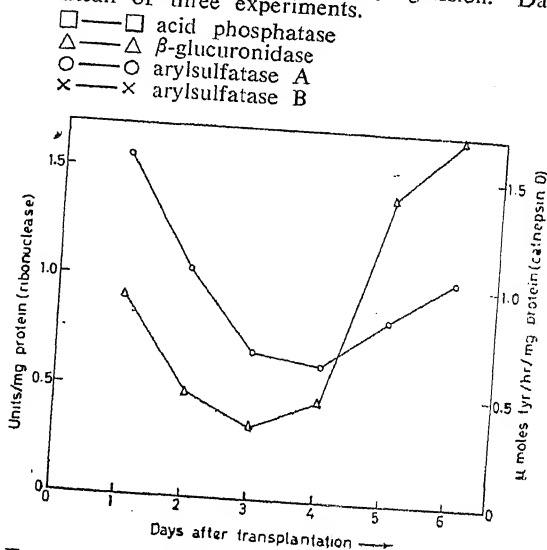


FIG. 3. Levels of acid ribonuclease and cathepsin D (free plus bound) in YAS during tumour progression. Data are mean of three experiments.

TABLE I

Percentage free activity\* of lysosomal enzymes during growth of Yoshida ascites sarcoma

Enzymes	% Free activity				
	Days				
	1	2	3	4	5
Acid phosphatase ..	12.00	8.20	6.00	7.80	20.00
Acid ribonuclease ..	6.00	5.28	4.32	8.91	10.00
$\beta$ -Glucuronidase ..	6.50	6.41	3.78	4.52	7.83
Arylsulfatase A ..	3.00	0.80	0.93	1.26	3.29
do. B ..	2.87	0.72	0.83	1.73	4.21
Cathepsin D ..	20.09	18.28	11.00	22.61	29.20

Data are mean of three experiments.

\* Free activity was calculated by the formula  $F/B + F \times 100$ , where 'F' is free activity in cytosol, and 'B' is the bound activity in the lysosomal fraction.

new hosts. Parry and Ghadially<sup>13</sup> reported an increase in lysosomes during tumour growth and their rupture during the terminal phase.

## ACKNOWLEDGEMENT

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# CYTOLOGICAL STUDIES IN NORMAL AND MUTAGEN TREATED STRAINS OF TRITICALE (TRITICALE HEXAPLOIDE, LART)

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**C**YTOGENETIC investigation in many strains of 6 x triticales have demonstrated significant differences in meiotic instabilities, frequency of aneuploids and kernel fertility. Sporadic attempts have been made to increase the fertility level and quality of 6 x triticales, which is superior in seed and fertility characteristics to the 8 x forms but still not as good as wheat<sup>1-3</sup>. Ruebenbaver and Nalepa<sup>4</sup> have, however, demonstrated that exposure to ionizing radiation can lead to the isolation of mutants with higher seed fertility and increased winter hardiness in triticales. Our main objective in the present study was to develop some 6 x triticales lines which will have meiotic stability, pollen viability and kernel fertility comparable to wheat.

We treated three strains of 6 x triticales, viz., Arm. 130, PC 186 and BC 245 with gamma-rays (5, 10, 15 Kr), EMS (0.15%, 0.30% and 0.45%) and gamma irradiation plus EMS in combination (5 Kr + 0.15% EMS, 10 Kr + 0.15% EMS, and 15 Kr + 0.15% EMS) to see whether

mutagen treatment is helpful in improving the reproductive behaviour. Normal and mutagen treated seeds were grown in the field in Rabi, 1972. Meiosis was studied in both normal and mutagen treated M<sub>1</sub> populations. The young spikelets were fixed in a freshly prepared mixture of absolute alcohol and acetic acid (3 : 1) for 24 hr and finally stored in 70% alcohol. The anthers were then squashed in acetocarmine and meiosis was studied in detail. The frequency of univalents was observed at first metaphase, frequency of lagging chromosome and of chromatin bridges at first anaphase and frequency of micronuclei in tetrads. Pollen fertility was recorded as count of fertile pollen seen as completely filled, round and deeply stained with acetocarmine under microscope. Percent kernel setting was estimated for each treatment after counting the number of spikelets per spike. One hundred kernel weight in gm was also recorded.

The two strains Arm 130 and B C 245 were found sensitive to 5, 10, 15 Krs gamma irradiation.

tion in terms of percent irregular cells and mean univalents per cell observed at first metaphase (Table I.) Two mutagen treatments, viz., 0.15% anaphase as compared with the control and other mutagen treatments (Table III). The percent pollen viability and kernel setting in 0.15% EMS

TABLE I

*Chromosome analysis in the three strains of triticale at metaphase I*

Metaphase I	Control	5 Kr	10 Kr	15 Kr	0.15% EMS	0.30% EMS	0.45% EMS	5 Kr +0.15% EMS	10 Kr +0.15% EMS	15 Kr +0.15% EMS
Number of cells examined										
Arm 130	120	137	140	162	132	138	141	172	162	124
PC 186	96	106	98	121	110	86	117	132	119	98
BC 245	156	167	148	150	146	132	128	156	159	137
Number of irregular cells										
Arm 130	38.8	52.3	56.1	68.2	28.1	59.6	59.7	31.8	70.7	52.8
PC 186	25.4	31.1	27.7	33.8	27.7	23.3	31.1	29.0	25.7	21.9
BC 245	67.3	75.9	72.8	72.3	28.3	65.4	61.6	25.2	75.5	64.1
Percent irregular cells										
Arm 130	32.4	38.2	40.1	42.1	21.3	43.2	42.4	18.5	43.7	42.6
PC 186	26.5	29.4	28.3	28.0	25.2	27.2	26.6	22.0	21.6	22.4
BC 245	43.2	45.5	49.2	48.2	19.4	49.6	48.2	16.2	47.5	46.8
Mean univalents per cell										
Arm 130	0.98	1.10	1.23	1.12	0.82	1.24	1.16	0.62	1.19	1.12
PC 186	0.82	0.85	0.90	0.98	0.80	0.92	0.96	0.75	0.72	0.78
BC 245	1.37	1.42	1.48	1.38	0.78	1.32	1.27	0.58	1.16	1.22

EMS and 5 Kr gamma irradiation + 0.15% EMS were found to reduce the meiotic instability as evidenced by the low percentage of irregular cells and relatively low mean univalents per cell at first metaphase in Arm 130 and BC 245. Other treatments, viz., 0.30% EMS, 0.45% EMS, 10 Kr + 0.15% EMS, 15 Kr + 0.15% EMS seemed to increase the percent irregular cells and mean univalents per cell when compared with the control. The third strain PC 186 did not show any significant effect due to mutagen treatments over control. It is interesting to note that in 0.15% EMS and 5 Kr + 0.15% EMS treated plants, the percent irregular cells and mean laggard number per cell at first anaphase decreased significantly as compared to the control and other mutagen treatments (Table II). The same two treatments further exhibited some reduction in percent irregular cells and mean laggards number per cell at second

and 5 Kr + 0.15% EMS treatments were greater in Arm 130 and BC 245 strains (Table IV). There did not seem to be any marked effects over control of mutagen treatment on the PC 186 strain in regard to pollen viability and kernel setting. The 100 kernel weight was found increased in case of 0.15% EMS and 5 Kr + 0.15% EMS treated plants, and the results of other treatments were in keeping with the data on pollen viability and kernel setting (Table V).

In this study both percent irregular cells and mean univalents per cell at first metaphase or mean laggards per cell at first anaphase were used as criteria to identify the strains for their meiotic instability. Out of nine mutagen treatments, two treatments, viz., 0.15% EMS and 5 Kr + 0.15% EMS seemed to improve meiotic stability in two strains of triticale as their PMC exhibited relatively low percent irregular cells, low mean number



TABLE II

*Frequency of laggards at anaphase I in both control and mutagenically treated populations in triticale*

Anaphase I	Control	5 Kr	10 Kr	15 Kr	0.15% EMS	0.30% EMS	0.45% EMS	5 Kr +0.15% EMS	10 Kr +0.15% EMS	15 Kr +0.15% EMS
Number of cells examined										
Arm 130	210	185	205	187	212	189	207	185	208	195
PC 186	180	176	210	185	210	178	189	178	182	232
BC 245	235	210	230	167	200	210	176	204	168	242
Number of irregular cells										
Arm 130	84.4	76.8	93.8	86.4	56.6	89.7	96.8	35.9	101.0	97.9
PC 186	56.1	58.4	68.6	59.0	63.8	57.3	59.3	44.1	48.4	77.4
BC 245	66.7	62.2	70.4	49.7	45.8	63.2	57.3	33.2	52.4	84.7
Percent irregular cells										
Arm 130	40.2	41.5	45.8	46.2	26.7	47.5	46.8	19.4	48.6	50.2
PC 186	31.2	33.2	32.7	31.9	30.4	32.2	31.4	24.8	26.8	33.4
BC 245	28.4	29.6	30.6	29.8	22.9	30.1	32.6	16.3	31.2	34.6
Mean laggards per cell										
Arm 130	0.84	0.89	0.92	0.93	0.32	0.95	0.88	0.24	0.97	0.98
PC 186	0.68	0.71	0.70	0.73	0.58	0.74	0.69	0.43	0.58	0.64
BC 245	0.49	0.54	0.53	0.51	0.24	0.35	0.29	0.38	0.47	0.48

TABLE III

*Frequency of laggards at anaphase II in both control and mutagenically treated populations in triticale*

Anaphase II	Control	5 Kr	10 Kr	15 Kr	0.15% EMS	0.30% EMS	0.45% EMS	5 Kr +0.15% EMS	10 Kr +0.15% EMS	15 Kr +0.15% EMS
Number of cells examined										
Arm 130	280	275	210	265	280	210	185	195	210	263
PC 186	210	265	189	175	180	190	192	186	148	150
BC 245	195	185	200	210	205	260	262	265	280	270
Number of irregular cells										
Arm 130	84.5	89.1	77.0	101.7	48.7	82.3	71.0	29.6	89.0	116.2
PC 186	42.0	73.6	49.5	45.5	40.1	55.8	59.1	36.8	43.2	46.8
BC 245	70.1	69.1	77.8	77.2	57.8	101.0	105.0	21.9	114.2	105.8
Percent irregular cells										
Arm 130	30.2	32.4	36.7	38.4	17.4	39.2	38.9	15.2	42.4	44.2
PC 186	24.8	27.8	26.2	26.0	22.3	29.4	30.8	19.8	29.2	31.2
BC 245	36.5	37.4	38.9	36.8	28.2	38.9	40.1	18.3	40.8	39.2
Mean laggards per cell										
Arm 130	0.72	0.78	0.81	0.86	0.40	0.90	0.85	0.34	0.72	0.78
PC 186	0.50	0.62	0.64	0.68	0.42	0.58	0.52	0.41	0.60	0.58
BC 245	0.58	0.67	0.62	0.66	0.32	0.42	0.38	0.28	0.42	0.54

TABLE IV

Pollen viability and kernel setting in both control and mutagenically treated populations in triticale. Pollen viability data represent average of 10 plants having 200 microscopic field per treatment. Data on kernel setting represent average counts of 50 primary spikes per treatment

	Control	5 Kr	10 Kr	15 Kr	0.15% EMS	0.30% EMS	0.45% EMS	5 Kr +0.15% EMS	10 Kr +0.15% EMS	15 Kr +0.15% EMS
Pollen viability (%)										
Arm 130	85.4	89.0	73.6	72.5	92.3	75.5	58.3	90.0	76.4	64.5
PC 186	98.0	86.8	72.4	67.0	95.0	83.4	53.8	74.5	81.0	59.0
BC 245	82.2	84.2	75.5	70.0	94.2	82.6	81.7	96.7	75.2	58.5
Kernel setting (%)										
Arm 130	65	68	65	60	70	54	56	75	46	47
PC 186	80	78	74	74	75	70	80	80	70	71
BC 245	72	70	65	66	80	56	52	89	62	63

TABLE V

Mean 100 kernel weight in grams  
Data represent mean  $\pm$  S.E. of ten samples  
per treatment

Treatment	Arm 130	PC 186	BC 245
Control	2.77 $\pm 0.30$	3.84 $\pm 0.31$	3.10 $\pm 0.12$
5 Kr	2.90 $\pm 0.23$	3.08 $\pm 0.24$	3.00 $\pm 0.07$
10 Kr	2.55 $\pm 0.22$	3.39 $\pm 0.20$	3.06 $\pm 0.05$
15 Kr	2.76 $\pm 0.43$	3.29 $\pm 0.20$	3.04 $\pm 0.04$
0.15% EMS	2.78 $\pm 0.12$	3.68 $\pm 0.10$	3.58 $\pm 0.10$
0.30% EMS	2.50 $\pm 0.25$	3.10 $\pm 0.15$	3.20 $\pm 0.30$
0.45% EMS	2.78 $\pm 0.28$	3.69 $\pm 0.23$	3.32 $\pm 0.21$
5 Kr + 0.15% EMS	2.62 $\pm 0.33$	3.74 $\pm 0.25$	3.20 $\pm 0.16$
10 Kr + 0.15% EMS	2.50 $\pm 0.18$	2.90 $\pm 0.30$	3.12 $\pm 0.13$
15 Kr + 0.15% EMS	2.47 $\pm 0.13$	2.93 $\pm 0.11$	3.00 $\pm 0.07$

of univalents, and low mean laggards. Swaminathan<sup>5</sup> observed that the variability in the number of kernels per spike was much greater in 20 Kr gamma irradiation and 0.20% EMS treated population of some promising 6x triticales as compared to the control. The results of the present study also reveal greater variability with regard to pollen viability, kernel setting and mean 100 kernel weight in gamma-rays and EMS treated population of triticale strains. Triticales characteristically exhibit some degree of sterility which has generally

been attributed to meiotic abnormalities including incomplete pairing and asynchronous chromosome disjunction<sup>6</sup>. It is, however, suggested that as the meiotic irregularities are polygenically controlled, the mutagen at lower doses, for example 0.15% EMS as in this study, may be producing specific alterations at chromosomal DNA level for the improvement of incomplete pairing and asynchronous chromosome disjunction. At higher doses of gamma-rays or EMS, macromutational events including nonspecific alterations at chromosomal DNA level leading to greater variability can be expected. The possibility that low doses of mutagen treatment may be responsible to bring about some dependence between meiotic stability and kernel fertility is attractive but it demands that we learn more about the operational mechanism(s) causing meiotic instabilities and low kernel setting in triticale. It is further suggested that cytological stability and high kernel fertility can be attained in 6x triticale following vigorous progressive selection in the progenies of mutants selected for high stability and fertility.

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## LETTERS TO THE EDITOR

## CRYSTALLOGRAPHIC STUDY OF

 $\text{ZnMn}_{1-x}\text{Cr}_x\text{FeO}_4$  SPINELS

X-ray crystallographic study of the system  $\text{ZnMn}_{1-x}\text{Cr}_x\text{FeO}_4$  has been carried out for  $x$  varying from 0 to 1. The lattice constants of the compounds show that the tetragonal spinel phase ( $c > a$ ) at  $x = 0$  changes to cubic spinel structure at  $x = 0.5$ .

$\text{XY}_2\text{O}_4$  compounds with spinel structure have been extensively studied, as they exhibit interesting electrical and magnetic properties, which are controlled by the nature of ions, their charge and site distribution amongst 8 tetrahedral (A) and 16 octahedral (B) sites. In normal spinels X and Y are divalent and trivalent cations which occupy A and B sites respectively. In the case of normal spinels having diamagnetic X-ions like Zn, Cd, and Mg, etc. A-B interaction is absent<sup>1</sup> and hence the physical properties are mostly governed by the B-B interactions. Gradual replacement of ions of one type by another is found to yield a series of compounds whose crystal structure and other physical properties show interesting variation. Such mixed compounds can, indeed, be regarded as solid solutions of iso-structural members. In the case of mixed compounds of members belonging to different crystal systems the lattice is gradually distorted for different concentrations of the compounds, the degree of distortion changing with amount of one of the ions. Several<sup>2-5</sup> workers have studied solid solutions of spinels by replacing ions at A- and/or B-sites. O'Keeffe<sup>6</sup> has substituted  $\text{Fe}^{3+}$  for  $\text{Mn}^{3+}$  in  $\text{ZnFe}_2\text{O}_4$  at B-sites. Robbins and Darcy<sup>7</sup> have replaced  $\text{Mn}^{3+}$  ions at B-sites by  $\text{Cu}^{2+}$  and  $\text{Ge}^{4+}$  in  $\text{ZnCu}_x\text{Ge}_x\text{Mn}_{2-2x}\text{O}_4$ . However, spinels with three transitional elements at the B-sites have not been studied in detail. It is, therefore, of interest to study such a system so as to obtain a better understanding of the interactions amongst the transitional metal ions at the B-sites.

The compounds  $\text{ZnMn}_{1-x}\text{Cr}_x\text{FeO}_4$  with  $x$  having values 0, 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0 have been synthesised by the oxide method<sup>8</sup> by intimately mixing under acetone the oxides  $\text{ZnO}$ ,  $\text{Mn}_2\text{O}_3$ ,  $\text{Cr}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$  of A.R. grade in the molar proportion  $2 : 1 - x : x : 1$ . The mixture was dried in air and pressed into pellets under 5000 p.s.i. using polyvinyl acetate as a binder. The pellets were first heated slowly upto  $300^\circ\text{C}$  for 3 hours in an electric furnace to evaporate the binder. They were finally fired at  $900^\circ\text{C}$  for about 70 hours in a Pt-boat

and subsequently cooled slowly at the rate of  $100^\circ\text{C}$  per hour. X-ray diffraction patterns of the compounds, powdered to 300 mesh size, were obtained on 114.6 mm Debye-Scherrer camera using filtered copper radiation. Formation of the spinels was taken to be complete as the lines of the reacting oxides were absent in the patterns obtained. All patterns showed a single phase of spinel structure. Lattice parameters calculated from the observed  $d$ -values are included in Table I. The lattice parameters of  $\text{ZnMnFeO}_4$  are found to agree with values reported earlier<sup>6,9</sup>.

TABLE I

Lattice parameters of  $\text{ZnMn}_{1-x}\text{Cr}_x\text{FeO}_4$ 

$x$	Symmetry	Lattice constants in Å		$C/a - 1$	$(a^2c)^{1/3}$ in Å
		$a$	$c$		
0	T	8.30	8.73	0.052	8.44
0.2	T	8.33	8.39	0.007	8.35
0.4	T	8.33	8.36	0.003	8.34
0.5	C	8.29	8.29	0.0	8.29
0.6	C	8.31	8.31	0.0	8.31
0.8	C	8.35	8.35	0.0	8.35
1.0	C	8.44	8.44	0.0	8.44

T=tetragonal.

C=cubic.

Table I shows that the tetragonal distortion is considerably reduced on replacement of  $\text{Mn}^{3+}$  ions by  $\text{Cr}^{3+}$  ions at B-sites. This is predominantly an effect of a large decrease in  $c$  value of the tetragonal lattice (Fig. 1.1). On further replacement of  $\text{Mn}^{3+}$  by  $\text{Cr}^{3+}$  ions the distortion is gradually reduced and finally it disappears at  $x = 0.5$ . It is well known that the amount of  $\text{Mn}^{3+}$  required to produce co-operative bulk distortion<sup>10,11</sup> depends on the nature of other ions. O'Keeffe<sup>6</sup> has found that  $\text{Mn}^{3+}$  causes such a distortion in  $\text{ZnMn}_x\text{Fe}_{2-x}\text{O}_4$  at  $x = 0.75$ . Irani *et al.*<sup>12</sup> in their investigations on solid solutions of zinc and manganese manganites with different alluminates, ferrites and chromites, etc., observed that the critical fraction of  $\text{Mn}^{3+}$  required to cause tetragonal distortion is 0.6, i.e., 60% of B-sites should be occupied by  $\text{Mn}^{3+}$  ions. Robbins and Darcy<sup>7</sup> have shown that  $\text{Mn}^{3+}$  ions should occupy 50% of the B-sites in  $\text{ZnCu}_x\text{Ge}_x\text{Mn}_{2-2x}\text{O}_4$  to produce macroscopic distortion. In spite of the fact that both  $\text{Cu}^{2+}$  and  $\text{Mn}^{3+}$  ions on B-sites produce tetragonal distortion, the above amount of  $\text{Mn}^{3+}$  in  $\text{ZnCu}_x\text{Ge}_x\text{Mn}_{2-2x}\text{O}_4$  is more than that required to produce a similar distortion

in  $\text{ZnMn}_x\text{Fe}_{2-3x}\text{O}_4$ . The corresponding fraction of B-sites occupied by  $\text{Mn}^{3+}$  ions in  $\text{ZnMn}_x\text{Fe}_{2-3x}\text{O}_4$  is small, i.e., 37.5%. In the present series of compounds only 25% of B-sites need be occupied by  $\text{Mn}^{3+}$  to produce distortion of the cell. This low amount of  $\text{Mn}^{3+}$  necessary to produce lattice distortion may be attributed to the presence of transitional metal ions at B-sites. Further, the  $\text{Fe}^{3+}$  ion with configuration  $3d^5$  is spherically symmetric while  $\text{Cr}^{3+}$  with  $3d^3$  configuration is likely to be asymmetric. It appears that such an asymmetry of  $\text{Cr}^{3+}$  enables  $\text{Mn}^{3+}$  to co-operate at a lower concentration and cause a distortion of the lattice.

A plot of  $\sqrt[3]{a^2c}$  against  $x$  (Fig. 1.2) shows that the lattice volume decreases as  $\text{Mn}^{3+}$  is replaced by  $\text{Cr}^{3+}$ . The unit cell has a minimum volume at

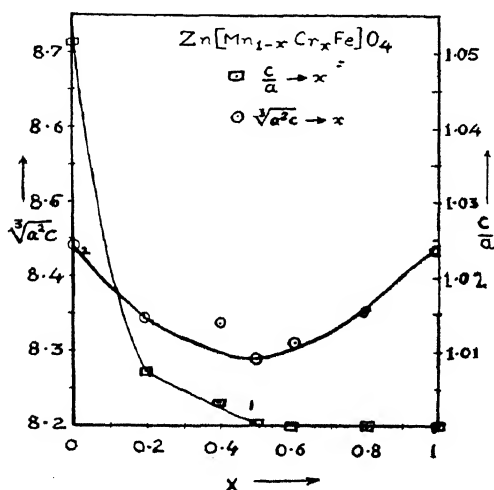


FIG. 1.1.  $c/a \rightarrow x$ .  
FIG. 1.2.  $\sqrt[3]{a^2c} \rightarrow x$ .

the composition  $\text{ZnMn}_{0.5}\text{Cr}_{0.5}\text{FeO}_4$  at  $x=0.5$  which is in contrast with a generally observed linear variation of  $\sqrt[3]{a^2c}$  with  $x$ . It is not possible to account for this minimum at present. However, a study of electrical and magnetic properties may provide useful information to explain the minimum.

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## TRICYANOBIS ( $\gamma$ -PICOLINE) NITROSYLIRON (II)

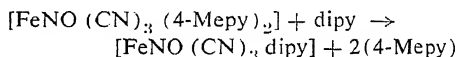
Hexa-coordinated, substituted cyano nitrosyl iron (II) has been prepared using  $\gamma$ -picoline as a substituent. Infra-red spectrum of this compound indicates that the complex is of *cis* variety having  $C_{2v}$  symmetry and there is stronger F-NO back bonding than in nitroprusside ion.

In continuation of our interest to prepare same hexa-coordinated iron (II) nitrosyl complexes<sup>1</sup>, which are rare<sup>2</sup>, we now report the study of tricyanobis ( $\gamma$ -picoline) nitrosyl iron (II).

$\gamma$ -PICOLINIUM salt of nitroprusside was prepared by the reaction of stoichiometric amounts of  $\gamma$ -picolinium hydrachloride and sodium nitroprusside dihydrate in methanol, digesting the mixture to precipitate NaCl, and evaporating the solution to a minimum volume followed by drying in vacuum, after removing NaCl by filtration. About 10.0 g of the brown salt was taken in a boat and heated at 145° in an atmosphere of  $\text{CO}_2$  for about five hours when the dehydrocyanogenation reaction ceased and a pale greyish-brown mass was obtained. It was cooled under  $\text{CO}_2$  atmosphere and then washed with methanol and dried in vacuum.

Found: Fe, 16.30; N, 23.8%;  $[\text{FeNO}(\text{CN})_3(4\text{-Mepy})_2]$  requires Fe, 15.95; N, 24.0%.

The complex is sparingly soluble in alcohols and water and insoluble in other common solvents. Alcoholic solution of  $\text{AgNO}_3$  does not give any immediate precipitation but on standing, precipitation of  $\text{AgCN}$  occurs slowly. In aqueous or alcoholic medium, dipyrldyl gives immediately a red colour which is presumed to be due to the reaction given below. The dipyrldyl complex has been characterised recently using different synthetic routes<sup>1</sup>.



Infra-red spectrum of the  $\gamma$ -pic. substituted complex shows two bands due to  $\text{CN}^-$  stretching modes and one very strong band due to NO stretching in addition to the bands of coordinated  $\gamma$ -picoline (Table I).

TABLE I  
Infra-red spectra of 4-Mepy and  $[\text{FeNO}(\text{CN})_3(4\text{-Mepy})_2]$

4-Mepy	$[\text{FeNO}(\text{CN})_3(4\text{-Mepy})_2]$	assignments
742 s	722 m	co-ordinated
823 vs	802 s	4-Mepy bands
1015 vs	1022 s	"
1066 m	1235 m	"
1235 s	..	..
1436 w	1508 s	"
1460 w	1625 vs	"
1610 vs	1640 s	"
	1907 vs	$\nu(\text{NO})$
	2148 m	$\nu(\text{CN})$
	2187 vs	$\nu(\text{CN})$

vs=very strong, s=strong, m=medium, w=weak. The spectra were recorded in Nujol.

The point group of the molecule should be  $C_g$ . The number of i.r. bands expected on this basis due to  $\nu(\text{CN})$  is three. However, the appearance of only two CN bands suggest that there is not much 'difference' between the nitrogen atoms of NO and  $\gamma$ -picoline and the local symmetry  $C_{3v}$  could be assigned for the molecule as observed for some substituted tricarbonyl compounds, where N-donor ligands are the substituents<sup>4</sup>. The appreciable reduction in  $\nu(\text{NO})$  indicates that Fe-NO back bonding is greater in this complex than that in the starting nitroprusside complex.

The authors wish to thank Mr. R. N. Singh, Principal, T.D. College, Jaunpur, for providing facilities; Professor G. B. Singh of Banaras Hindu University for infra-red spectral measurements and Professor R. P. Rastogi of Gorakhpur University for encouragement.

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## STRUCTURE OF THE MYRICETIN GLUCOSIDE FROM THE FLOWERS OF *CALOPHYLLUM INOPHYLLUM*

In the course of the chemical study of the polyphenolic constituents present in the andraecium of the flowers of *Calophyllum inophyllum*, Sankar-subramanian and Nair<sup>1,2</sup> isolated a new myricetin glucoside the structure of which was assigned as the 7-glucoside. This was based on the colour reactions as well as the spectral properties of the glucoside. Complete methylation of the glucoside followed by acid hydrolysis was reported to give a partial pentamethyl ether of myricetin which was found to be different from 3-hydroxy-5, 7, 3', 4', 5'-pentamethoxyflavone<sup>3</sup> as well as 5-hydroxy-3, 7, 3', 4', 5'-pentamethoxyflavone<sup>4</sup>. Since the partial methyl ether, obtained from the glucoside of myricetin, yielded 3, 4, 5-trimethoxybenzoic acid on alkali fission, the constitution of the partial methyl ether was assigned as 7-hydroxy-3, 5, 3', 4', 5'-pentamethoxyflavone and hence the glucoside was considered to be myricetin-7-glucoside<sup>2</sup>. However, the earlier workers did not synthesise 7-hydroxy-3, 5, 3', 4', 5'-pentamethoxyflavone and make a direct comparison with the compound prepared from the myricetin glucoside.

The synthesis of 7-hydroxy-3, 5, 3', 4', 5'-pentamethoxyflavone has now been done as a result of which a direct comparison with the partial methyl ether, obtained from the natural product, could be made. The synthesis makes use of 5, 7-dihydroxy-3, 3', 4', 5'-tetramethoxyflavone<sup>5</sup> which is prepared from  $\omega$ -methoxyphloracetophenone, tri-O-methylgallate anhydride and sodium tri-O-methylgallate (Allan-Robinson condensation). Partial tosylation<sup>6</sup> of the dihydroxy-tetramethoxyflavone, using toluene-*p*-sulphonyl chloride and anhydrous potassium carbonate in acetone medium, yields 5-hydroxy-3, 3', 4', 5'-tetramethoxy-7-tosyloxyflavone (yellow needles, m.p. 172–73°). Subsequent methylation of the tosyl ester by the dimethyl sulphate-acetone-potassium carbonate method gives 3, 5, 3', 4', 5'-pentamethoxy-7-tosyloxyflavone (pale yellow needles, m.p. 105–07°), which on subsequent alkaline hydrolysis with boiling 10% aqueous sodium carbonate solution and acidification produces 7-hydroxy-3, 5, 3', 4', 5'-pentamethoxyflavone (m.p. 250–51°). It does not give any characteristic colour with alcoholic ferric chloride but gives a wine-red colour with magnesium and hydrochloric acid as well as a red colour with zinc and hydrochloric acid. The melting point of 7-hydroxy-3, 5, 3', 4', 5'-pentamethoxyflavone, now prepared by synthesis, is not found to be depressed on admixture with the sample of the partial methyl ether of myricetin (m.p. 250–51°), obtained earlier from the myricetin glucoside<sup>2</sup>. The identity

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between the two samples is also established by paper as well as thin layer chromatography. The U.V. spectrum of the synthetic compound in ethanol as well as in the presence of sodium acetate is identical with that reported earlier<sup>2</sup>. This confirms that the structure of the myricetin glucoside, isolated from the andraecium of the flowers of *Calophyllum inophyllum* is in fact the 7-glucoside.

Our thanks are due to Professor S. Sankar-subramanian of the Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-6, for his interest in this work and to the University Grants Commission, New Delhi, and the Madurai University for the award of a Research Scholarship to one of us (S. M. K.).

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#### A STUDY OF THE A.F.O. OXIDATION OF 2-HYDROXY- $\alpha$ -METHYL FURFURYLIDENE ACETOPHENONES

ALGAR-FLYNN-OYAMADA (A.F.O.) oxidation of 2-hydroxy furfurylidene acetophenones<sup>1</sup> revealed that 2-hydroxy-furfurylidene acetophenones, possessing a 6-substituent gave rise to 2-hydroxy-2-furylidene-coumaran-3-ones, while those lacking it, furnished 3-hydroxy-2-(2-furyl)-chromones. The latter compounds may have been formed by the oxidation of the intermediate 3-hydroxy-2-(2-furyl)-chromanones. A similar suggestion was made in the case of flavonoids also<sup>2,3</sup>. To confirm the view a study of the A.F.O. oxidation of 2-hydroxy- $\alpha$ -methyl-furfurylidene acetophenones (I) has been made.

Several 4- or 5-substituted 2-hydroxy- $\alpha$ -methyl furfurylidene acetophenones (Chalcones) I (1-5) (Table I) have been prepared by the base catalysed condensation of the appropriate 2-hydroxy propiophenones and furfural. These chalcones have been oxidised with alkaline hydrogen peroxide both

at 0° and at elevated temperature 25°. The products of oxidation II (1-5) (Table I) have been found to be homogeneous on tlc with different solvent systems.

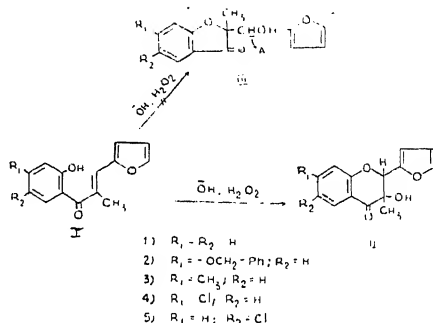


TABLE I

2-Hydroxy- $\alpha$ -methyl furfurylidene acetophenones	3-Methyl-3-hydroxy-2- (2-furyl)- chromanones
I. (1) Simple, 2, 4-DNP. 191°	II. (1) Simple—151°
(2) 4-Benzoyloxy—100°	(2) 7-Benzoyloxy—110°
(3) 4-Methyl—45°	(3) 7-Methyl—96°
(4) 4-Chloro—80°	(4) 7-Chloro—112°
(5) 5-Chloro—95°	(5) 6-Chloro—105°

The compound II (2) exhibited, in ir (KBr) absorptions at 3460  $\text{cm}^{-1}$  (free-OH) and 1690  $\text{cm}^{-1}$  ( $>C=O$  in six membered ring with conjugation with aryl group<sup>4</sup>), in UV (EtOH), at 240–245 nm and inflexion at 285–290 nm. In nmr, 60 MHz in  $\text{CDCl}_3$ , it gave absorptions,  $\delta=1.43$  (S, for 3-Me) (3 H), 3.80 (S, for 3-OH) (1 H), 5.05 (S, for  $\text{CH}_2$  of  $\text{C}_6\text{H}-\text{CH}_2-$ ) (2 H), 5.26 (S, for H-2)<sup>3</sup> (1 H) and several absorptions between 6.35 and 7.95 for aromatic protons (6 H). The presence of hydroxyl group has been confirmed by  $\text{D}_2\text{O}$  exchange studies. On the basis of the evidence, particularly that of ir absorption at 1690  $\text{cm}^{-1}$  and the signal in nmr, at  $\delta=5.263$ , the oxidation products have been formulated as 3-methyl-3-hydroxy-2-(2-furyl)-chromanones (II) (1-5). The alternate structure III for these compounds is eliminated on the ground that the signal due to proton-A, which should appear at  $\delta=4.963$ , and the carbonyl absorption<sup>4</sup> at 1710  $\text{cm}^{-1}$  in III are absent in the nmr and ir spectra of these compounds respectively.

All attempts to dehydrate these compounds with protonating acids like concentrated sulphuric acid and dehydrating agents such as phosphorous pentoxide in refluxing benzene have failed. This may be due to the cis relationship of H and OH in II. It is very likely that 2-(2-furyl) group takes up less crowded equatorial environment, resulting

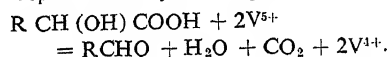
in axial and equatorial conformations for H and OH respectively.

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### KINETICS OF COPPER CATALYSED OXIDATION OF LACTIC ACID BY $V^{5+}$

OXIDATION of  $\alpha$ -hydroxy acids by  $V^{5+}$  in perchloric acid medium was first reported by Waters and co-workers<sup>1</sup>, who explained their results in terms of a C-C bond fission and the overall reaction was represented by the equation



The order of  $[V^{5+}]$  and  $[Hydroxy\ acid]$  was found to be one each, and the rate was found to be acid dependent increasing with increase in  $[H^+]$ . Also, it was pointed out that a soluble complex between the hydroxy acid and  $V^{5+}$  at higher concentration of hydroxy acid is likely<sup>2</sup>.

In the present work, the kinetic data on the oxidation of lactic acid (LA) in the presence and absence of  $Cu^{2+}$  ions which is known to form a complex with LA are presented and possible mechanisms discussed.

All chemicals used were of highest purity and wherever necessary further purification was done by standard methods. The rate of oxidation of LA was followed by quenching the reaction mixture at different intervals of time in a known excess of  $Fe^{2+}$  and titrating the unreacted  $Fe^{2+}$  against standard  $V^{5+}$  using barium diphenyl amino sulphate as indicator. The order of  $[V^{5+}]$  and  $[LA]$  were found to be one each in the absence of  $Cu^{2+}$  in sulphuric acid medium. Also, the stoichiometry of the reaction was found to be, two  $V^{5+}$  to one LA and the product identified as acetaldehyde by spot tests<sup>3</sup>. The stoichiometry did not change in the presence of  $Cu^{2+}$  ions.

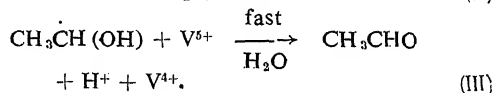
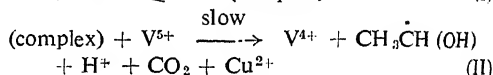
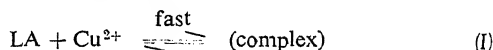
At constant ionic strength and  $[SO_4^{2-}]$  the pseudo first order rate constant  $k'$  (under conditions of  $[LA] \gg [V^{5+}]$ ) increased with increase in  $[Cu^{2+}]$  at 0.04 M sulphuric acid (Table I). Further, the order of LA in the presence of  $Cu^{2+}$  was less than

TABLE I

Effect of  $[Cu^{2+}]$  on  $k$  at low  $[H^+]$  and constant  $[SO_4^{2-}]$   
 $[V^{5+}] = 4 \times 10^{-3} M$ ;  $[LA] = 0.1 M$ ;  $[H^+] = 0.64 M$ ;  
temp. = 50° C.  $\mu = 0.4 M$

$[Cu^{2+}]$ m.l. <sup>-1</sup>	0.00	0.024	0.048	0.072	0.096
$k' \times 10^3$ min <sup>-1</sup>	1.31	1.38	1.51	2.37	2.63
$k_{Cu}/k_0$	..	1.05	1.15	1.81	2.00

unity ( $\sim 0.8$ ) suggesting complexation of LA with either  $V^{5+}$  or  $Cu^{2+}$ . Though  $V^{5+}$  is capable of forming a complex with LA at higher concentration<sup>2</sup> of LA and high  $H^+$ , in view of low concentration of LA and  $H^+$  used in the present work, it is probable that LA might form a complex with  $Cu^{2+}$  before oxidation by  $V^{5+}$ . The  $Cu^{2+}$ -LA complex could be a better reductant which incidentally explains the catalytic activity of  $Cu^{2+}$  ions. Such a mechanism was envisaged by Ram Reddy *et al.*<sup>4</sup> to explain the catalytic activity of  $Cu^{2+}$  ions in the oxidation of mannitol by  $Ce^{4+}$  in sulphuric acid medium. Hence, the probable mechanism could be written as:



The rate was found to increase with increase in  $[H^+]$  both in the presence and absence of  $Cu^{2+}$  (Table II). This may be explained as due to the existence of the following equilibria:

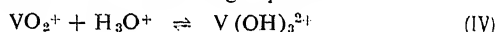


TABLE II

Effect of  $[H^+]$  at constant  $[SO_4^{2-}]$  and  $\mu$  on  $k$   
 $[V^{5+}] = 0.004 M$ ;  $[LA] = 0.1 M$ ;  $[Cu^{2+}] = 0.06 M$ ;  
temp. = 50° C.  $\mu = 0.844 M$

$[H^+]$ m.l. <sup>-1</sup>	0.025	0.05	0.075	0.100	0.125	0.200
$k_0 \times 10^2$	0.359	1.22	1.67	2.88	3.91	4.26
min <sup>-1</sup>						
$k_{Cu} \times 10^2$	0.921	2.12	2.48	3.57	4.72	4.95
min <sup>-1</sup>						
$k_{Cu}/k_0$	2.56	1.74	1.48	1.36	1.21	1.16

and that  $V(OH)_3^{2+}$  is the reactive species. The catalytic activity of  $Cu^{2+}$  ions was measured in terms of  $k_{Cu}/k_0$  ratio where  $k_{Cu}$  and  $k_0$  are the rate constants in the presence and absence of  $Cu^{2+}$  ions respectively. This ratio was found to decrease with increase in  $[H^+]$  (Table II). This could be explained if one assumes that complex formation between LA and  $Cu^{2+}$  results in a decrease of pH. The effect of  $[H^+]$  on this would be to decrease the concentration of complex and hence, the rate. Thus equilibrium (IV) and (I) oppose each other in

so far as the effect of  $[H^+]$  on the rate of oxidation of LA is concerned. The data in Table II indicates that though the rate constants in the presence or absence of  $Cu^{2+}$  increase with  $[H^+]$  their ratios  $k_{cu}/k_0$ , however, decrease indicating that formation of a copper-LA complex before oxidation by  $V^{5+}$  to be more probable. Also, the increase in  $k_{cu}/k_0$  values with increase in  $Cu^{2+}$  at constant  $[H^+]$  (Table I) gives further support to the proposed mechanism.

The activation energy and other thermodynamic parameters at low  $[H^+]$  (0.04 M) in the absence and presence of 0.06 M  $Cu^{2+}$  (in paranthesis) are as follows.  $\Delta E^\ddagger = 29.3$  (20.2) K.Cals  $mol^{-1}$   $\Delta H^\ddagger = 28.7$  (19.5) K.Cals  $mol^{-1}$ ;  $\Delta G^\ddagger = 14.4$  (14.2) K.Cals  $mol^{-1}$  and  $\Delta S^\ddagger = 44.2$  (16.6) e.u. The thermodynamic parameters show considerable decrease in  $\Delta S^\ddagger$  in the presence of  $Cu^{2+}$  suggesting a more rigid structure for the activated complex as could be expected if  $Cu^{2+}$ -LA complex is oxidised in the rate determining step. The decrease in the  $\Delta E^\ddagger$  value explains well the increase in the rate.

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## FLAVONOID CONSTITUENTS OF THE LEAVES OF CINCHONA LEDGERIANA

IN this communication we report the isolation of reynoutrin, delphinidin and chromatographic identification of kaempferol and quercetin from the leaves of *Cinchona ledgeriana*<sup>1</sup>.

**Reynoutrin.**—The air-dried leaves of *C. ledgeriana* were extracted with methanol and the concentrated extract was subjected to a preliminary clean up (from the waxy constituents and alkaloids) in the usual way. The residue (A) obtained in this manner was dissolved in water and extracted with ethyl acetate. The aqueous layer, after its saturation with sodium chloride, was thoroughly extracted with *n*-butanol. The *n*-butanol concentrate was enriched in flavonoid constituents by employing the counter current distribution technique. From the

resulting solution, solvent was distilled off under diminished pressure and the residue (B) was subjected to preparative TLC on silica gel, using ethyl acetate-acetic acid-water (13:3:3) as the developer solvent<sup>2</sup>. The major, pale yellow, band (visualizing agent, 1%  $AlCl_3$  in ethanol) on the chromatoplate was eluted with methanol and after a careful work-up, yielded pale yellow needles (10% aqueous methanol), m.p. 201–3°. It exhibited principal UV absorption bands at  $\lambda_{max}^{EtOH}$  258 (log  $\epsilon$  4.31), 360 nm (log  $\epsilon$  4.22). The compound reacted with  $FeCl_3$  (greenish brown coloration) and responded to Shinoda and Molisch's tests. The glycoside was identified as reynoutrin<sup>3,4</sup> by its acid hydrolysis to quercetin and xylose.

**Kaempferol and Quercetin.**—TLC on silica gel of the acid hydrolysate of residue (B), using toluene-ethyl formate-formic acid (5:4:1)<sup>5</sup>, gave two spots (UV light) corresponding to kaempferol and quercetin.

**Delphinidin.**—The alcoholic solution of residue (A) on acid hydrolysis, in the usual way, furnished a dark red solid—which responded to the colour reactions of anthocyanidins<sup>6</sup>. Preparative TLC (silica gel) of the solid, using ethyl acetate-formic acid-conc. HCl-water (55+6+1+8)<sup>7</sup> furnished delphinidin<sup>8</sup> ( $\lambda_{max}^{MeOH-HCl}$  549, 456 (sh), 370 and 265 nm; with  $AlCl_3$   $\Delta\lambda = 22$  nm; and had  $R_f$  0.33 (*n*-BuOH–2 N HCl, 1:1), and 0.55 (*m*-cresol–AcOH–5 N HCl, 1:1:1).

We wish to thank Dr. R. S. Kapil, Central Drug Research Institute, Lucknow, for his interest and Dr. S. K. Nigam, National Botanic Gardens, Lucknow, for kindly sending us the authentic samples of kaempferol and quercetin.

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# APIGENIN GLYCOSIDES FROM *THUNBERGIA FRAGRANS* AND *RUELLIA TUBEROSA*

In an earlier communication<sup>1</sup>, we reported the isolation of apigenin-7-O-glucuronide and other flavones from *Thunbergia grandiflora* and *Asystasia travancorica* (Acanthaceae) and observed that ours was the first report of occurrence of the flavone uronide in the Acanthaceae. Subsequently the isolation of apigenin glucuronide from *Ruellia prostrata* and *Barleria cristata* of the same family was reported by us<sup>2</sup>. As part of a survey of occurrence of flavone uronides in South Indian plants for biological studies and in continuation of our study of the flavonoids of the Acanthaceae, we have examined the flowers and leaves of *T. fragrans* and *R. tuberosa* and the results are recorded in brief.

## *T. fragrans*

The major crystalline compound isolated from the aq. alc. concentrate of the flowers of *T. fragrans* was found to be a sterol glycoside, m.p. 294–96°, which on hydrolysis with 2 N HCl yielded  $\beta$ -sitosterol (characterised by m.p. and m.m.p.,  $[\alpha]_D^{25}$ , acetyl derivative and co-TLC with an authentic sample) and D-glucose (identified by  $R_f$  and co-PC). Thus, the glycoside was identified as  $\beta$ -sitosterol-D-glucoside and the identity confirmed by direct comparison including co-TLC and superimposable I.R. spectrum of the glucoside acetate with an authentic sample.

The flowers and leaves contained two flavones, which were identified as apigenin-7-O- $\beta$ -D-glucuronide (major) and apigenin-7-O-rutinoside (minor) by m.p.,  $\lambda_{max}$ ,  $R_f$ , products of acid as well as enzyme hydrolyses and co-chromatography with authentic samples.

## *R. tuberosa*

The leaves, yellow buds and deep violet flowers of *R. tuberosa* were separately examined. The leaves contained only traces of apigenin and luteolin, while the flowers malvidin-3,5-diglucoside in appreciable quantity. The flower buds contained the maximum proportion of flavonoids yielding about 3% of apigenin-7-O-glucuronide; the other flavones were identified as apigenin-7-O-glucoside, apigenin-7-O-rutinoside and luteolin-7-O-glucoside.

Our reports of isolation of flavone glucuronides from the Acanthaceae lend biochemical support to the placement, along with it, of the Bignoniaceae, Labiatae, Schrophulariaceae and Verbenaceae under the same Natural Order Tubiflorae, known to contain flavone uronides<sup>3</sup>. The occurrence of significant quantity of apigenin-7-glucuronide in the buds and its disappearance in the petals are interesting from the point of study of glucuronide metabolism in plants<sup>4</sup>.

We thank Drs. L. Hörhammer and H. Wagner of the University of Munich for certain spectral data and Dr. T. N. C. Vedantham for comparison of our sterol glucoside with authentic  $\beta$ -sitosterol-D-glucoside.

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# COMPARATIVE STUDIES ON THE LETHAL AND MUTAGENIC EFFECTS OF FORMALDEHYDE AND HYDROGEN PEROXIDE IN *CHAETOMIUM AUREUM* CHIVERS

MUTAGENIC effects of formaldehyde<sup>1</sup> and hydrogen peroxide<sup>2,3</sup> have long been established on *Drosophila*<sup>1</sup> and *Neurospora*<sup>2,3</sup>. An attempt was made to study the mutagenic efficiency of these chemicals on *Chaetomium aureum*<sup>4</sup> with a view to isolate mutants for genetical investigations.

Treatments of 20, 30, 40, 60, 80, 100, 120 and 140 minutes with 0.1 molar formaldehyde (E. Merck) and hydrogen peroxide (F. Merck) were given to the ascospore suspensions at 26°C. After treatment the spores were washed thrice with sterile distilled water and the treated spores plated on P-Complete<sup>5</sup> medium having 0.7% sorbose. Survival per cent was determined after incubation at 30°C for five days. The average of 10 plates per treatment including the control was taken into consideration. Fries<sup>6</sup> total transfer technique was followed for the isolation of morphological and biochemical mutants. Auxanographic technique<sup>7</sup> was followed to determine the actual nutritional requirements of the probable biochemical mutants on the minimal medium\*. Cellulase activity of different mutants and wild strain was determined following the standard techniques<sup>8-10</sup>.

Table I shows that treatment with formaldehyde gave a sharp decline in the survival percentage. No biochemical mutant was induced but a few colonial and apigmented mycelial types of morphological mutants were obtained with 80, 100 and 120 minutes of treatment.

At 20 minutes treatment with hydrogen peroxide a sharp rise in viability of ascospores was noticed (Table I). Only a few inositol requiring biochemical mutants were obtained with 80 minutes of hydrogen peroxide treatment,

TABLE I  
*Lethal and mutagenic effects of formaldehyde and hydrogen peroxide on Chaetomium aureum*

Treatment in minutes	Average surviving ascospores ( $\times 10^6/\text{ml}$ )	Survival (%)	Colonies tested	Morphological mutants (%)	Biochemical mutants (%)	Total mutants (%)
<i>Formaldehyde</i>						
0 (Control)	26.70	100.00	1,000	—	—	—
20	22.69	84.34	1,000	—	—	—
30	12.44	46.59	1,000	—	—	—
40	4.43	16.59	1,000	—	—	—
60	1.42	5.33	1,000	—	—	—
80	0.76	2.86	1,000	0.10	—	0.10
100	0.28	1.06	1,260	0.39	—	0.39
120	0.01	0.32	1,260	0.15	—	0.15
140	0.0	0.02	1,000	—	—	—
<i>Hydrogen peroxide</i>						
0 (Control)	78.10	100.00	1,080	—	—	—
20	92.20	118.05	1,080	—	—	—
30	45.83	58.68	1,080	—	—	—
40	17.40	22.35	1,080	—	—	—
60	10.37	13.27	1,080	—	—	—
80	7.81	10.00	1,080	—	0.46	0.46
100	4.00	5.12	1,080	—	—	—
120	2.80	3.58	1,080	—	—	—
140	2.08	2.66	1,080	—	—	—

Temperature during treatment 26°C — absence.

It is interesting to note that mutants obtained with formaldehyde and hydrogen peroxide treatment lost their cellulase producing behaviour in contrast to the wild type. However, it is apparent from the data that lethality occurs in ascospores of *C. aureum* with the application of formaldehyde and hydrogen peroxide but the chemicals have no promising mutagenic effect on them.

Grateful thanks are due to Prof. S. M. Sircar, Director, and Prof. P. N. Nandi, Head of the Department of Microbiology, Bose Institute, for according facilities of work. The author also takes the opportunity to thank Shri K. L. Chaudhuri, Reader, Department of Microbiology, Bose Institute, for his guidance and help during the course of investigation.

Microbial Genetics

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\*  $\text{K}_2\text{HPO}_4$ —0.25 g;  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ —0.50 g;  $\text{NaNO}_3$ —2.0 g;  $\text{KH}_2\text{PO}_4$ —0.75 g;  $\text{KCl}$ —0.05 g; glucose (Analar)—15.0 g; Distilled water—1000 ml; pH—6.5; Purified Agar<sup>11</sup>—15.0 g.

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### CASTE DIFFERENTIATION IN THE PARER WASP *ROPALIDIA MARGINATA* (LEP.)

Wasps are remarkable amongst the higher social insects in exhibiting all stages of development of sociality from completely solitary to highly advanced colonial species with females clearly differentiated into reproductive and worker castes. The tropical paper wasp genus *Ropalidia* (= *Icaria*) occupies a particularly crucial stage in the evolutionary sequence of the development of social habit amongst the wasps<sup>1</sup>. Roubaud's<sup>2</sup> pioneering observations on the African members of *Ropalidia* suggest that this genus includes species with multiple egg-layers morphologically indistinguishable from the workers at a single colony. He reported that in two colonies of *R. guttatipennis* four out of six, and five out of seven females possessed functional ovaries. Apart from this sixty year old observation nothing seems to be known of caste differentiation in this interesting genus. The investigation reported here showed that the Indian species *R. marginata* is somewhat more advanced in caste differentiation, though belonging to the same crucial evolutionary stage.

*R. marginata* is a common Indian species which regularly builds its combs under the eaves and window sills of houses. The adult wasp is about 25 mm in length and about 60 mgm in weight. The number of adults in a colony ranges from two or three to seventy. Our observations suggest that the colony is founded by several females, and is perennial. We have been engaged in a study of the ecology and life-history of this species since October 1971 and this letter is the first report of this investigation.

During the course of this study we have collected twelve entire colonies of *R. marginata* from Poona. Adults at these colonies were sexed, weighed and dissected to ascertain the state of development of their reproductive organs. Apart from variation in size, there were no morphological differences amongst the females. There were, however, marked differences in the extent of development of the ovaries, the majority possessing totally atrophied ovaries. In each case one or two females possessed well-developed and obviously fully functional ovaries, while in a few cases upto four more females had moderately developed ovaries which may have been functional to some degree. Females with functional ovaries were always amongst the heavier individuals, often, but not always, being the heaviest wasp in the colony. Figure 1 presents the data on the weights and the state of ovarian development for four representative colonies.

It is clear that *R. marginata* resembles *R. guttatipennis* in the occurrence of a functional differen-

tiation of egg-layers and workers without any corresponding morphological distinction. However the caste differentiation in *R. marginata* has progressed further in that only a minority or often just one female possesses functional ovaries. This caste differentiation is presumably brought about through behavioural interactions. We have observed extensive food sharing at the *R. marginata* colonies. It may therefore be conjectured that workers expend more labour in food gathering but receive a disproportionately smaller share of the food. The ovaries of workers may then be atrophied due to this "nutritional castration". We hope that our further studies with individually marked wasps will enable us to test this hypothesis and lead to a solution of this problem.

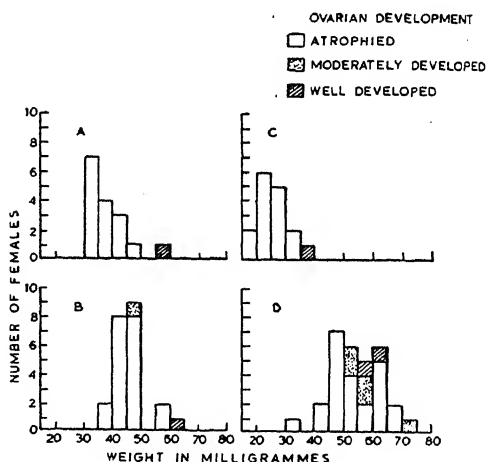


FIG. 1

We are grateful to Prof. O. W. Rishards of British Museum for identifying the specimens.

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**DISTRIBUTION OF SILVER IN GRANITIC GNEISSES NEAR NARSIPATNAM AREA, VISAKHAPATNAM DISTRICT, ANDHRA PRADESH**

SILVER is very infrequently determined in rocks and minerals as its concentration is so low (crustal abundance is 0.07 ppm), that it is usually impossible to determine even with sensitive spectrochemical procedures. While carrying out the trace elemental determinations on granitic gneisses and their silicates from Narsipatnam area of Visakhapatnam District by X-ray emission spectroscopy, the authors have detected spectral lines of silver at wavelength 3280 Å. Encouraged by these findings, determinations of silver in these rocks and silicates were made for the first time quantitatively using Perkin Elmer Model-303 Atomic absorption spectrophotometer whose detection limit for Ag is 0.1 ppm.

The Ag determinations carried out in silicates semiquantitatively using X-ray emission spectroscopy and in granitic gneisses by Atomic absorption spectrophotometer (quantitatively) are given (Tables I and II). Table I shows hypersthene amidst the silicates is having the highest concentration of Ag followed by garnet and then biotite. It is observed from Table II that orthopyroxene granitic gneisses show highest concentration of silver followed by orthopyroxene hornblende granitic gneisses, potash granitic gneisses and garnet-biotite granitic gneisses.

TABLE I

Sample No.	Name of the silicate	Silver in ppm
T11	Hypersthene	50
T22	Almandine	20
S10	do.	less than 20
D5	Biotite	less than 20
D2	do.	less than 20
S10	do.	less than 20

TABLE II

Sample No.	Name of the rock	Ag ppm	Ag/Cu
D1	Orthopyroxene granitic gneiss	13.9	0.07
T11	Orthopyroxene granitic gneiss	13.4	0.08
50M	Orthopyroxene hornblende granitic gneiss	13.2	0.07
D2	K feldspar granitic gneiss	12.9	0.03
D5	Biotite granitic gneiss	11.9	0.12
S16	Plagioclase granitic gneiss	10.9	0.22
60X	Garnet biotite granitic gneiss	8.8	0.04
T32	K feldspar garnet biotite granitic gneiss	8.3	0.04
B11	Garnet, biotite granitic gneiss	7.1	0.14

Geochemically silver occurs in nature mostly as sulphides with galena, chalcopyrite or as silver sulphide. As it has got more affinity,  $\text{Ag}^+$  can substitute in silicates for  $\text{K}^+$  among the common elements;  $\text{Ag}^{+2}$  may enter either  $\text{Ca}^{+2}$ , Na or  $\text{Fe}^{+2}$  lattices<sup>1</sup>.

Some of the granitic gneisses consisting of silver were polished, but no silver-bearing minerals were detected. However, opaques like chalcopyrite, pyrite and magnetite are present particularly in garnetiferous granitic gneisses. Hence it is probable that either (1)  $\text{Ag}^+$  is associated with  $\text{K}^+$  in the K-feldspar granitic gneisses or (2)  $\text{Ag}^{+2}$  substitute  $\text{Fe}^{+2}$  of the hypersthene in the orthopyroxene granitic gneisses or (3)  $\text{Ag}^{+2}$  substitutes  $\text{Fe}^{+2}$  in pyrites and chalcopyrites in garnetiferous granitic gneisses. Further as Ag atoms show great mobility during high temperatures<sup>1</sup>, the Ag content may be entrapped in the irregular cracks of garnets as adsorbed ions, during high grade metamorphism.

The rocks having pyroxenes, garnets and feldspars represent highest grade of metamorphism in the region. During metamorphism, chalcopyrite, pyrite and magnetite were introduced into these rocks and perhaps the pneumatolytic, hydrothermal solutions brought in Ag which get redistributed in various metamorphic and metasomatic minerals.

It is significant to find that not even the traces of silver is found in the calcium rich rocks consisting of minerals like scapolite, andradite, grossularite, anorthite and clinopyroxene even though it is known that  $\text{Ag}^{+2}$  may enter  $\text{Ca}^{+2}$  site. Hence the concentration of silver in the rocks can be accounted as replacement of  $\text{Fe}^{+2}$  of iron bearing silicates and sulphides in these gneisses. The Ag/Cu ratio of the rocks show neither sympathetic nor antipathetic relationship to the acidity of the rocks.

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### EXTRAPHARYNGEAL THYROID FOLLICLES IN A CATFISH *HETEROPNEUSTES FOSSILIS*\*

THYROID follicles have been reported to occur at various non-pharyngeal sites like gut, spleen, liver, ovary, kidney, heart, brain and choroid gland of the eye in fishes<sup>1-5</sup>. At their non-pharyngeal locations, they are termed as extrapharyngeal or heterotopic thyroid follicles. In the present investigation, their presence is observed in the mesonephros of *Heteropneustes fossilis*.

*Heteropneustes fossilis* is a freshwater catfish which is found abundantly in the ponds and lakes of Bhopal. It is a bottom dwelling carnivorous fish with accessory respiratory organs. The fish for the present purpose were purchased from the fish-market of Bhopal where they are largely marketed daily in living condition. The mesonephros of twelve adult specimens have been examined to investigate the presence of thyroid tissue in them. For this, the usual method of sectioning the tissue at 6  $\mu$  thickness and the routine process of double staining with Delafield's Hematoxylin and Eosin have been employed.

Out of twelve, only one fish possessed the extrapharyngeal thyroid follicles. They are found to be located in the middle region of the mesonephric kidney, in the neighbourhood of blood vessels. They are solitary in condition and very few in number. In the fish under investigation, the authors could have counted only four such follicles. They are variable in size but all of them are circular in their outline. They are surrounded externally by a distinct epithelial covering. The colloid, which they contain, is homogeneous and acidophilic in nature. The colloid does not possess vacuoles in it (Fig. 1).

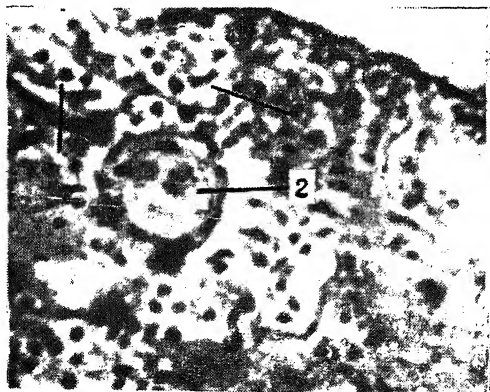


FIG. 1. Photomicrograph of a cross section passing through the middle region of mesonephros, showing the presence of an extrapharyngeal thyroid follicle.  $\times 450$ . 1, Blood vessel; 2, Extrapharyngeal thyroid follicle; 3, kidney tubule.

Extrapharyngeal thyroid follicles have been reported to be functional in several fishes<sup>2,3,4</sup>. The histological picture of these follicles, in *H. fossilis*, suggests their inactiveness. Their nearness to the blood vessels can be taken as granted that they might have migrated along them to their heterotopic position, a view also held by some recent authors<sup>1,3,4</sup>.

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### FOLLICULAR ATRESIA IN SOME BATS

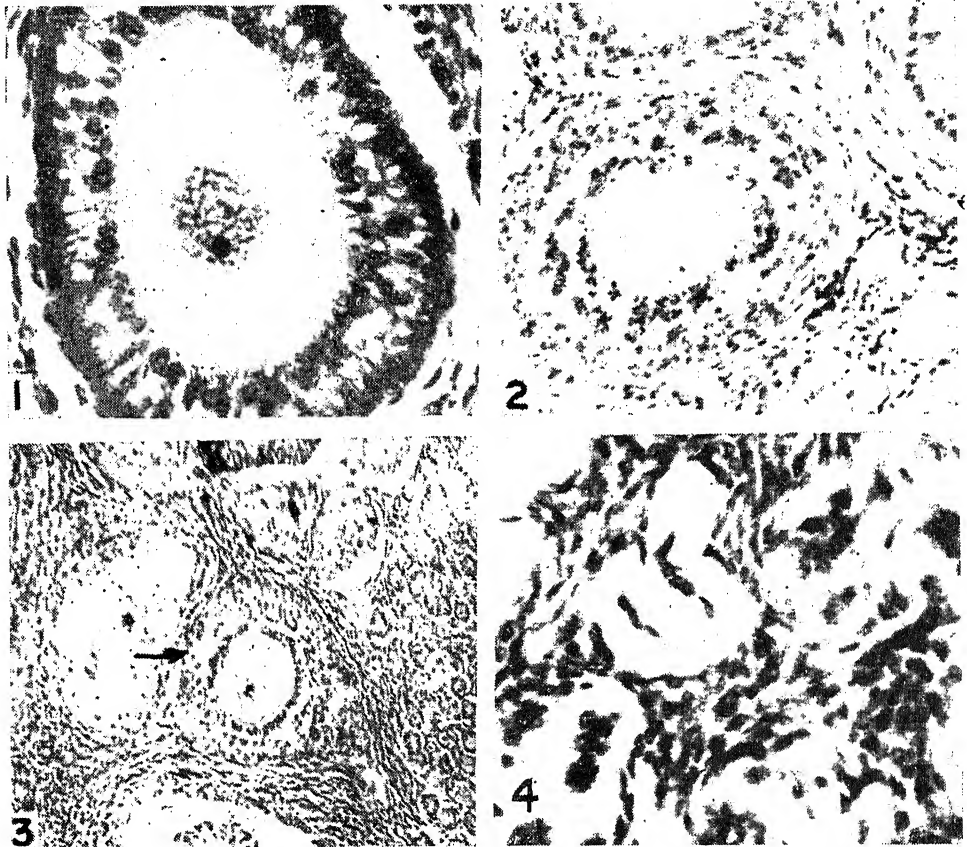
DURING the course of the study of the seasonal changes in the ovaries of several species of Indian bats, it was observed that follicular atresia occurs at any of the stages of the development of the follicle in these animals, and that the sequence of atretic changes varies considerably among the different types of follicles.

The present note is based on the examination of numerous ovaries of two species of megachiroptera, namely, *Rousettus leschenaulti* and *Pteropus giganteus* (Pteropidae), and five species of microchiroptera, namely, *Megaderma lyra lyra* (Megadermatidae), *Rhinolophus rouxi* (Rhinolophidae), *Hipposideros speoris* (Hipposideridae), *Pipistrellus ceylonicus* and *Pipistrellus mimus mimus* (both belonging to family Vespertilionidae). *Pteropus*, *Megaderma*, *Rhinolophus*, *Hipposideros* and *Pipistrellus ceylonicus* breed once in a year; *Rousettus* breeds twice in quick succession in a year and *Pipistrellus mimus mimus* breeds throughout the year.

In all the species studied here, atresia of the primordial follicles (follicles in which the oocyte is surrounded by a single layer of flat satellite cells) occurs extensively both during the prepubertal life, and during the non-breeding season and pregnancy in the adult animal. In all these cases the first sign of atresia is noticed in the oocyte whose cytoplasm

becomes vacuolated and the nucleus becomes pycnotic. Soon, the nuclear membrane appears to rupture releasing its contents into the cytoplasm of the oocyte. During advanced stage of atresia the entire oocyte becomes irregular in outline and gets separated from the satellite cells whose nuclei become pycnotic. The oocyte degenerates followed by the degeneration of the satellite cells. Numerous empty areas where follicles were earlier present were noticed in the ovaries of *Roussettus*. Such empty spaces were, however, not noticed in the ovaries of the other bats studied here.

surrounded by a number of layers of follicle cells), and vesicular follicles (follicles with one or more antral spaces), the follicular atresia can commence either with the follicle cells or with the oocyte. When atresia begins with the follicle cells, the first sign of atresia is the development of vacuoles in the cytoplasm of these cells (Fig. 1). This is followed by the break-down of cell boundaries of these cells so that the pycnotic nuclei of these cells appear to be freely floating in a mass of cytoplasm surrounding the oocyte (Fig. 2). In vesicular follicles, the granulosa cells lining the antral cavity show



FIGS. 1-4. Fig. 1. Photomicrograph of an atretic bilaminar follicle of *Roussettus leschenaulti*. Note the presence of vacuoles in the cytoplasm of the follicle cells,  $\times 560$ . Fig. 2. An early multilaminar follicle of *Rhinolophus rouxi* showing the presence of numerous pycnotic nuclei lying in a mass of the cytoplasm of the cells,  $\times 140$ . Fig. 3. A part of the ovary of *Pteropus giganteus giganteus* showing an atretic follicle (arrow). Note the distorted shape of the oocyte,  $\times 110$ . Fig. 4. A part of the ovary of *Pteropus giganteus giganteus* showing the crumpled zona pellucida in empty follicle at the final stage of degeneration,  $\times 560$ .

In the unilaminar follicles (follicles with oocytes surrounded by one layer of cuboidal or low columnar follicle cells), bilaminar follicles (follicles with oocytes surrounded by two layers of follicle cells), multilaminar follicles (follicles with oocytes

degenerative changes first, while the cumulus cells and the granulosa cells lying adjacent to the membrana propria remain intact. The liquor folliculi becomes foamy progressively from the peripheral region to the centre of the antrum.

When atresia commences with the oocyte, the granulosa cells remain intact while the oocyte loses its spherical shape and becomes irregular in outline (Fig. 3). Vacuoles which are formed in the cytoplasm of the oocyte increase progressively in size and number. The atretic changes then extend to the granulosa cells which also develop vacuoles and finally disappear.

In both types of atresia the zona pellucida is the last structure to disappear and often remains for a considerable period as a crumpled membrane (Fig. 4) lying in empty follicles bounded by a few thecal cells.

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#### A NOTE ON THE PRESENCE OF *STRONGYLOIDES PAPILLOSUS* AND *NEOASCARIS VITULORUM* LARVAE IN THE MILK OF BUFFALOES

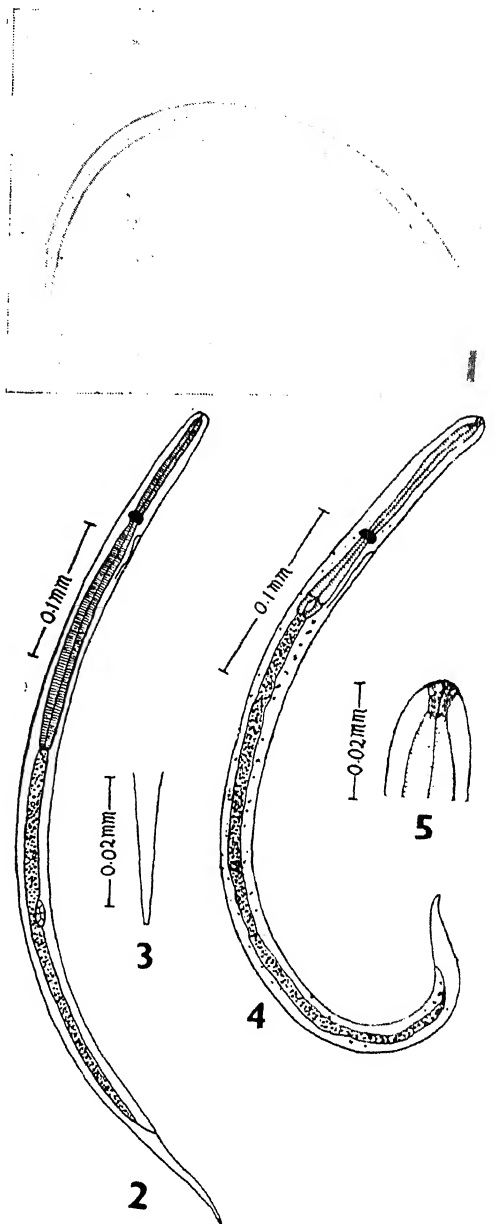
Of late, several workers from abroad have recorded the presence of nematode larvae in the milk of nursing dams<sup>2-7</sup>.—*Neoascaris vitulorum* (cattle, buffalo)<sup>4, 6, 7</sup>, *Strongyloides ransomi* (pig)<sup>3</sup>, *Ancylostoma caninum* (dog)<sup>5</sup> and *Uncinaria lucasi* (seals)<sup>2</sup>. These reports indicate that milk as well may be an important source of infection to the suckling animals particularly in case of nematodes where somatic migration/prenatal infection takes place. As far as could be ascertained there is no report from our country on *trans-mammary* passage of nematode larvae.

From September 1973 through March 1974, several samples of fresh milk (2 oz at a time) from 10 buffaloes of the college farm, Mathura, were examined 1–38 days post-parturition as per method followed by Tongson<sup>6</sup>. Two types of larvae, assignable to *S. papillosus* and *N. vitulorum*, were recovered. Brief descriptions of the larvae along with measurements (in microns) are given below.

*Strongyloides papillosus* (Figs. 1–3)

In all, 6 larvae were recovered from the milk samples of 3 buffaloes, on 7, 11, 17, 19, 22 and 23-day post-parturition. The filariae form larvae, with a head diameter of 10, measured 560–627 in length and 20–23 in maximum width. Nerve ring and excretory pore were situated at 81–88 and 92–95 distance respectively from the anterior end. The funnel-shaped buccal cavity opened into a filariae form oesophagus measuring 248–255 in length and 10–13 in maximum width. The 24 × 6.8 sized genital primordium was situated at 350–380 distance behind the anterior end. The tapering tail, with a slightly notched tip, measured 80–95 in length.

Except for a slightly larger size-range, the larvae, recovered from the milk in the present study, conformed to the description of Chauhan, Bhatia and Pande<sup>1</sup> for the third infective stage of *S. papillosus*.



FIGS. 1–5. Fig. 1. Microphotograph. Figs. 2–5. Camera lucida drawings. Fig. 1. Microphotograph of *S. papillosus* larva recovered from the milk of a buffalo ( $\times 190$ ). Fig. 2. Complete larva of *S. papillosus*. Fig. 3. Part of tail magnified. Fig. 4. Complete larva of *N. vitulorum* recovered from the milk of a buffalo. Fig. 5. Anterior end, magnified.



from buffaloes. The identification was further confirmed as the faeces of the young calves became positive for the eggs of *S. papillosus*. The finding of *S. papillosus* larvae in the milk of buffaloes is, obviously, a first record.

*Neoascaris vitulorum* (Figs. 4 and 5)

In all, 3 larvae were recovered from the milk samples of 2 buffaloes on 8 and 24-day post-parturition. Larvae, with a bluntly rounded anterior and gradually tapering posterior end, measured 425–510 in length and 14–20 in maximum width. The 3 lipped condition was apparent at the anterior end. Nerve ring and excretory pore were situated at 78–81 and 81–90 distance respectively from the anterior end. The oesophagus measured 136–146 in length and 10–12 in maximum width. The tail measured 38–42 in length. The faeces of these suckling calves later became positive for the eggs of *N. vitulorum*.

Tongson<sup>6</sup> encountered two types of *N. vitulorum* larvae (third stage) in the milk of buffaloes in Philippines: (i) Smaller sized found in majority, measured 442–628  $\mu$  and (ii) A few larger sized measured 1150–1580  $\mu$  in length. Our material resembled the smaller sized *N. vitulorum* larvae described by Tongson<sup>6</sup>. Present report, on the occurrence of *N. vitulorum* larvae in the milk of buffaloes, is evidently a first record from India.

Thanks are due to Principal of the College for the facilities provided.

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#### DRY ROT OF GINGER CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID.

THE cultivation of ginger (*Zingiber officinale* Roxb.) is seriously handicapped by the incidence of soft rot or rhizome rot disease caused by *Pythium* sp. in several tracts of Kerala. The fungus causes pre-emergence and post-emergence rotting of rhizomes during the South-West monsoon from June to September. Under Kasaragod conditions

the total rainfall received during June to September 1972 was 2521.1 mm with a mean temperature range of 23.0–30.8°C and a mean relative humidity range of 90.4–95.0%. During the course of investigations on soft rot disease, incidence of another type of rot in mature rhizomes of ginger was noticed from late October onwards till the time of harvest, i.e., February. This rot was found to persist even during storage. During this period (October 1972–February 1973) the total rainfall received was 236.1 mm with a mean temperature range of 20.0–32.7°C and a mean relative humidity range of 87–93%. A perusal of the literature shows that no similar disease has been recorded in ginger and hence reported here.

Affected plant under field conditions shows slight yellowing of leaves in the initial stages, and in advanced stages the whole plant presents a blighted appearance. Unlike in soft rot disease, the base of the stem does not decay and as such the plant does not snap away at the collar region on a slight pull. During advanced stages of the disease, the rhizomes appear shrunken and the inner tissues show discolouration and start disintegrating. Later the inner core of the rhizome shows dark sclerotia of a fungus adhering loosely to the fibrous tissues. The affected tissues do not exhibit any wet rot. Such rhizomes shrink and dry up. Hence this disease is termed as dry rot (Fig. 1).

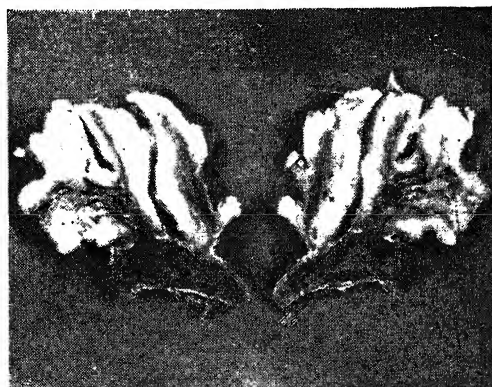


FIG. 1. Longitudinal section of dry rot-affected ginger rhizome.

Two fungi, viz., *Fusarium* sp. and another sclerotial form were isolated consistently from affected rhizomes. On potato sucrose agar medium *Fusarium* sp. sporulated abundantly while the sclerotial form produced large number of sclerotia. Both in culture and infected tissues the sclerotia appeared as black, minute, anastomosed dark hyphae, the interior of which is light brown to dark in colour with thick walled cells. Sclerotia were either



globose, oval, oblong, or irregular in shape and  $20-200 \mu \times 28-172 \mu$  in size.

For pathogenicity tests fresh rhizomes of ginger variety 'Maran' were sown in beakers of 500 ml capacity containing garden soil mixed with oat meal inoculum at 3:1 ratio. Both the isolates were tested individually and in combination. The moisture content was maintained at 50% water holding capacity of the soil. The inoculated beakers were incubated at room temperature ( $28-30^\circ \text{C}$ ). In eight days rhizomes inoculated with the fungus producing sclerotia showed darkening and shrinking to a depth of 5-10 mm. Similar result was obtained when both the isolates were inoculated in combination; but no synergistic action was noted. Pathogenicity tests with *Fusarium* sp. alone gave negative results. Thin hand sections of the infected rhizomes showed both inter and intracellular hyphae. Reisolations from the deeper portion of the affected tissue yielded sclerotial form only.

Under field conditions the rhizomes, which are injured either mechanically or by pests like rhizome weevil grubs, showed extensive colonisation by the sclerotial fungus. Therefore it appears that in majority of cases initial injury of rhizomes predisposes them to the fungal invasion, and subsequent colonisation.

The organism has been identified as *Macrophomina phaseolina* (Tassi) Goid., by Dr. Mordue of C.M.I., London (IMI. 172541). Only the sclerotial form of the fungus was produced in culture maintained in this laboratory. *M. phaseolina* has not so far been recorded as a causative organism of dry rot of ginger. The only dry rot that has been reported from ginger is the one caused by *Diplodia natalensis* (Wilson and Balagopal, 1971). Thus this appears to be a new record on ginger. Detailed studies on the fungus and its control measures are under way.

Thanks are due to Shri M. C. Nambiar, Project Co-ordinator, All-India Co-ordinated Spices and Cashewnut Improvement Project and to Shri K. V. Ahamed Bavappa, Director, Central Plantation Crops Research Institute, Kasaragod, for their encouragement, and to the Director, C.M.I., London, for the identification of the fungus.

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# FIRST RECORD OF SPIDER MITE *TETRANYCHUS LUDENI* ZACHER TRANSMITTING DOLICHOS ENATION MOSAIC VIRUS

DOLICHOS enation mosaic virus (DEM V), first reported on *Dolichos lablab* L. in 1948<sup>1</sup>, is a virulent leguminous strain of tobacco mosaic virus with a wide host range. It is easily transmitted only by sap. So far it is not known to be carried either through seed or by any vector<sup>1-4</sup>. During studies on DEM V infection in some legumes such as *D. lablab*, *Phaseolus mungo* L., *P. aureus* Roxb., and *Glycine max* (L.), Merr., at the S.V. Agricultural College, Tirupati, in 1972-73, it was observed that even the healthy controls exhibited foliar symptoms of DEM V. Further tests on the local lesion host cluster bean (*Cyamopsis psoraloides* DC)<sup>5</sup> confirmed the presence of the virus also in the healthy series. A vector was, therefore, suspected and it was observed that only webbing red spider mites were seen both in the healthy and infected plants of these crops. Critical mite transmission studies as suggested by Slykhuys<sup>7</sup> were, therefore, undertaken with DEM V on field bean (*D. lablab*) var. Local Red in insect proof nylon cages in the glasshouse with appropriate controls.

In the first experiment, the nymphs/adults of these mites actively feeding on DEM V-infected *D. lablab* leaves (Fig. 3) were carefully collected and transferred to 8-day old field bean plants at 30 mites per leaf on both the primary leaves. After allowing them to feed for 4 days, they were killed by spraying miticide. Mite damage was evident as white specks on the leaves. These plants exhibited conspicuous stunting (average height reduction of 38.2%) as compared to the control (Fig. 1) 30 days after transferring mites. Eleven out of 13 plants developed typical foliar abnormalities and symptoms (Fig. 2 a to c). These symptoms were identical with those observed on *D. lablab* by sap inoculation with DEM V<sup>1</sup>. The saps extracted individually from all the 13 plants, in turn, produced systemic infection on field bean and local lesions on cluster bean (1.1 to 140.3 lesions per leaf) thereby proving that this mite acts as a vector for the transmission of DEM V.

In the next experiment, the nymphs/adults of the mite on DEM V-infected *D. lablab* plants collected carefully were macerated in glass mortar with pestle at 500 mites per ml of distilled water. This mite extract when rubbed on young field bean and cluster bean plants with 'celite' as abrasive<sup>4</sup> produced characteristic DEM V symptoms while controls receiving extracts of similar mites collected from healthy plants did not reveal any symptoms. This time, the average number of lesions per leaf worked out to 4.6 indicating pro-

bably low virus concentration in the mite extract. However, the result clearly reveals that the mites collected from infected plants are viruliferous and carry DEMV.

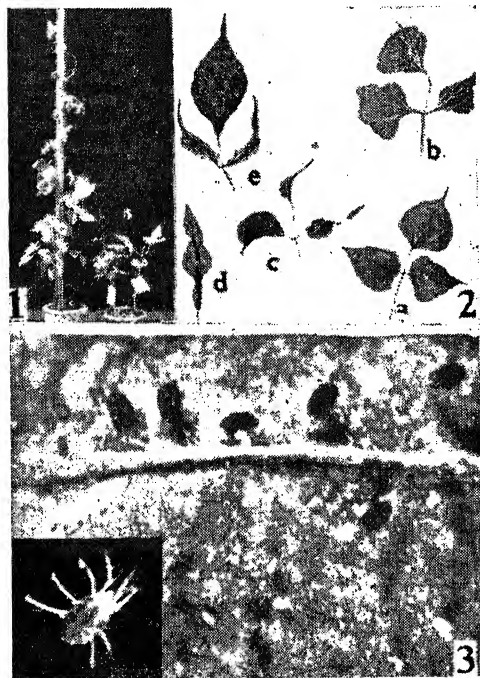


FIG. 1. *Dolichos lablab*: Healthy control (Left); Plants stunted consequent to transfer of mites from DEMV-infected plants (Right).

FIG. 2. Foliar abnormalities and symptoms on trifoliate leaves of *D. lablab* consequent to transfer of viruliferous mites (a) healthy leaf, (b) vein clearing and malformation, (c) and (d) enations on the under surface of leaves and (e) mosaic, puckering and malformation.

FIG. 3. Larvae, nymphs and adults of the mite on the under surface of leaf of *D. lablab* and a single adult mite,  $\times 32$  (Inset).

In another test, the spider mites from healthy plants were transferred to young DEMV-infected *D. lablab* plants (maintained free of mites earlier) for acquisition feeding of the virus for a week. These mites were later transferred to another set of 8 days old healthy plants, allowed to feed on them for 4 days and then killed. These *D. lablab* plants exhibited systemic infection of DEMV.

In the final experiment, small pots containing just emerged healthy field bean plants were placed inside big pots containing well grown DEMV-infected *D. lablab* plants infested with spider mites to give them chance of natural mite infestation. The mites migrated to the new ones and the new plants developed systemic symptoms of DEMV in 3-4 weeks.

Thus, the above findings prove beyond doubt that the nymphs/adults of the red spider mite (Fig. 3 inset) transmit DEMV. This has since been identified as *Tetranychus ludeni* Zacher. Only one tetranychid mite *T. telarius* (L.) has so far been proved to transmit potato virus Y though some others are suspected<sup>2,5,6</sup>.

The results presented are significant in three respects: (i) this is the first report of a vector transmitting DEMV, (ii) this gives positive proof of a second *Tetranychus* sp., viz., *T. ludeni* transmitting a plant virus and (iii) in view of wide host range for both DEMV and the tetranychid mite, timely mite control in legumes assumes importance.

Further studies on transmission are under progress.

I am deeply indebted to Dr. G. P. ChannaBasavanna, Professor and Head of Entomology, University of Agricultural Sciences, Bangalore, for identification of the mite. I am thankful to Shri M. Achutarama Rao and Shri B. Nagalingam for technical assistance and to Dr. J. Subbayya, Professor and University Head of the Department of Plant Pathology for critical perusal of the manuscript.

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#### MYROTHECIUM AND ALTERNARIA LEAF SPOTS OF COTTON IN SOUTH INDIA

*Myrothecium roridum* Tode ex Fr. causes a leaf spot of cotton in the Punjab and Haryana where it affects a number of cotton varieties (*Gossypium hirsutum*)<sup>1,2</sup>. The disease has not hitherto been reported on cotton in South India. Specimens of leaf spot affecting Hybrid-4 cotton sent from Gujarat in 1971 were referred to the same disease by the senior author. About the same time stray cases of the disease appeared on Hybrid-4 plants raised at Kovilpalayam near Coimbatore from seed obtained from Gujarat. The disease was not encountered in this area in 1972. Following the rains in October, 1973, a serious outbreak occurred in Coimbatore Taluk in all the important cotton growing centres and severe leaf damage was

sustained by the important varieties belonging to the species *G. hirsutum* and *G. barbadense*, including MCU 5, Sujata and the hybrids Hybrid-4, Varalaxmi and C.B.S. 156. The outbreak lasted till the end of January, 1974.

A feature of this season was the negligible incidence of the leaf spot of cotton caused by *Alternaria macrospora* Zimm. in areas where *Myrothecium* leaf spot occurred, although the disease had occurred as severe epiphytotics between November and February in each of the four preceding years causing alarming defoliation. This disease is characterised by smaller (0.2 to 1 cm diam.), nearly circular to irregular, dark brown spots with concentric ridges giving a target board appearance. There is no abscission of the spots as happens in the case of *Myrothecium* leaf spot.

The interrelationship between the two pathogens was studied. Strong conidial suspensions from pure cultures of the two pathogens separately and together were sprayed on the leaves of variety MCU 5 (*G. hirsutum*). Inoculated plants were placed in a humid chamber for 24 hr. Three days later circular leaf spots typical of those caused by *M. roridum* developed on all leaves sprayed either with *M. roridum* alone or with a mixture of *M. roridum* and *A. macrospora* while spots caused by *A. macrospora* appeared after 4 to 5 days in large numbers only on leaves sprayed with *A. macrospora* alone and were relatively sparse or absent on leaves inoculated with a mixture of the two fungi (Table I). In the latter case, *Alternaria* spots were seen in areas of the leaf well removed from spots caused by *Myrothecium*. Similar results were obtained with Sujata (*G. barbadense*) and other varieties.

TABLE I

No. of spots caused by *M. roridum* and  
*A. macrospora*

Inoculum	No. of spots per leaf	
	<i>Myrothecium</i>	<i>Alternaria</i>
<i>M. roridum</i> ..	4 to 6	..
<i>A. macrospora</i> ..	..	10 to 14
<i>M. roridum</i> plus <i>A. macrospora</i> ;	4 to 7	0 to 3

*In vitro*, the two fungi did not show marked antagonism which appears to be a feature of the parasitic phase of these facultative parasites.

The encouragement to the investigation given by Dr. V. Santhanam, Project Coordinator, is appreciated.

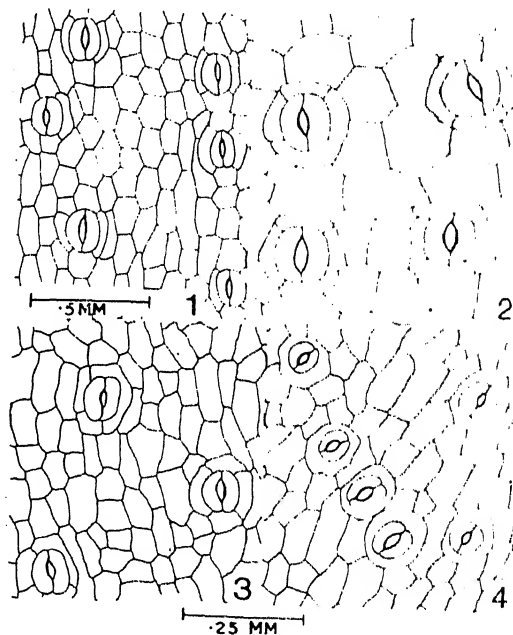
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## ORGANISATION OF STOMATAL COMPLEX IN SOME ORCHIDACEAE

EARLIER reports show that the stomata in Orchidaceae are of anomocytic type, i.e., they have two guard cells without any subsidiary cells<sup>1-4</sup>. However, the authors while studying the organisation of stomatal complex in Orchidaceae have observed subsidiary cells in several members, viz., *Rhynchosstylis retusa* Blume, *Vanda cristata* Lindl., *Calanthe brevicornu* Lindl., *Coelogyne ovalis* Lindl., *C. cristata* Lindl., *Bulbophyllum odoratissimum* Lindl. and *Pholidota articulata* Lindl. var. *griffithii* K & P.



Figs 1-4. Figs. 1, 2. Epidermal peels of *Vanda cristata* and *Bulbophyllum odoratissimum* respectively. Figs. 3, 4. Epidermal peels from young and old leaves respectively of *Pholidota articulata* var. *griffithii*. Note in Fig. 4 dissolved anticlinal walls of subsidiary cells.

The leaves are hypostomatic but for *Calanthe brevicornu* where they are amphistomatic. The stomata are of tetracytic type<sup>5</sup> in all the species mentioned above. They are surrounded by four

TABLE I

No.	Name of species	Place of collection	Average size of stomata* in $\mu$	Frequency cm/sq*	Stomatal index
1.	<i>Bulbophyllum odoratissimum</i> Lindl.	Shillong	42.6 × 42.6	11,360	14
2.	<i>Calanthe brevicornu</i> Lindl.	Dhakuri Pass, Almora	Upper surface 82.2 × 74.5 Lower surface 86.2 × 74.5	480 5,920	2 15
3.	<i>C. cristata</i> Lindl.	Mallagarkha, Pithoragarh	99.4 × 78.1	7,200	16
4.	<i>Coelogyne ovalis</i> Lindl.	Shillong	106.5 × 92.3	7,040	17
5.	<i>Pholidota uriculata</i> Lindl. var. <i>griffithii</i> K. & P.	Mallagarkha, Pithoragarh	56.8 × 71	8,480	10
6.	<i>Rhynchostylis ratusa</i> Blume	Kapkote, Almora	99.4 × 56.8	4,075	11
7.	<i>Vanda cristata</i> Lindl.	Mallagarkha, Pithoragarh	85.2 × 71	11,200	6

\* Mean of 10 values.

subsidiary cells which are alike in *Coelogyne ovalis*, *C. cristata*, *Bulbophyllum odoratissimum* and *Pholidota articulata*. They lie around guard cells in all four directions (Fig. 2). However, in *Rhynchostylis retusa*, *Vanda cristata* and *Calanthe brevicornu* two of the subsidiary cells are smaller that lie at the end of guard cells, while the remaining two are lateral to guard cells (Fig. 1). Occasionally stomata with three subsidiary cells have also been observed in *Pholidota articulata* (Fig. 3). In addition to normal tetracytic type of stomata in *Rhynchostylis retusa*, *Vanda cristata* and *Calanthe brevicornu* occasionally some stomata have been observed where subsidiaries surrounding the guard cells hardly differ from the other epidermal cells.

While the anticlinal walls of the subsidiary cells show partial dissolution in mature stomata of *Coelogyne*, they are completely dissolved in *Pholidota* (Fig. 4). Thus in these species the mature stomata are not linked with subsidiary cells and they appear like "floating stomata" of some ferns<sup>6</sup>.

The average size of stomata, stomatal index and stomatal frequency in members investigated is given in Table I.

The presence of tetracytic stomata in Orchidaceae may be of some significance in understanding evolutionary relationships of the family.

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#### A NEW FRUIT ROT OF BANANA IN INDIA

WHILE making a survey of post-harvest diseases of some fruits, a severe rotting of banana fruit (*Musa sapientum* Linn.) "Harichal" variety was observed in the local fruit markets in the months of July–September, 1973.

Under natural conditions the infection appeared in the middle or tip portions of the fruit as olive brown coloured spots. The spots extended longitudinally both towards the proximal and the distal ends of the fingers. These spots gradually increased in size and ultimately entire surface of the fruit displayed a clove brown or mummy brown colour due to coalescence of a number of spots. With the advancement of the infection, the fruit became more pulpy and oozed out a juice emitting foul odour. Later on, a white cottony growth of the fungus consisting of profusely branched hyphae appeared over the rotted tissue of the fruit (Fig. 1). The fungus associated with the rotting was isolated on Asthana and Hawker's 'A' medium by the usual method and was identified as an isolate of *Fusarium moniliforme* Sheld. The pathogenicity of the fungus was confirmed by inoculating both injured and uninjured fruits and subsequently isolating the same organism on the culture media. The pathogen appeared to be highly pathogenic as even uninjured fruits when sprayed with its spore suspension showed rotting within few days. The morphological characters of the fungus were as follows:

Hyphae hyaline, 2–5  $\mu$  in diameter, white to light orange colour in culture, septate, septa at a

distance of  $20-40\mu$ ; Chlamydospores absent; microconidia produced in chains, remain connected loosely, one-celled, sometimes two-celled, oval or spindle-shaped. one-celled conidia  $4-12 \times 2-4\mu$  (average  $8.6 \times 3.4\mu$ ) and two-celled conidia  $10-18 \times 2-4\mu$  (average  $13.6 \times 3.6\mu$ ) in size; macroconidia usually absent.

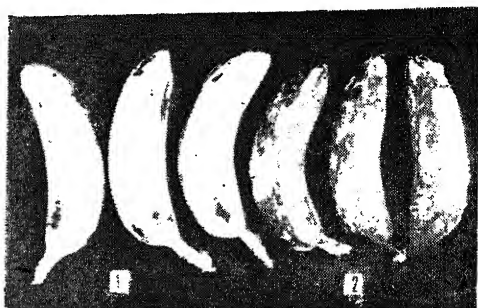


FIG. 1. Showing healthy (1) and diseased (2) banana fruits.

The culture has been deposited in the Botany Department, University of Allahabad, Allahabad and C.M.I., Kew, England (IMI 178496).

This disease of banana fruit has not been reported from India. Hansford<sup>1</sup> reported the present fungus to be responsible for the tip rotting of banana fruit in Uganda. Wollenweber<sup>2</sup> found *F. moniliforme* var. *minus* on decaying fruits of banana in association with *Pseudonectria musae* Hochapfel from America. Wardlaw<sup>3</sup> found the present pathogen responsible for tip-rotting of immature cavendish banana fruits in Trinidad. Chorin and Joffe<sup>4</sup> reported it to be associated with the rotting of banana fruits in Israel.

Authors are grateful to Dr. A. Johnston, Director, C.M.I., Kew, for the identification of the culture and Prof. D. D. Pant, Head of the Botany Department, University of Allahabad, Allahabad, for providing laboratory facilities.

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#### OCCURRENCE OF THE PERFECT STAGE OF *ALTERNARIA TENUIS* NEES OF THE LEAVES OF *MARSILEA QUADRIFOLIA* L.

IN December 1973, the author observed a serious leaf spot disease of *Marsilea quadrifolia* L. growing in the puddles of Bihar University campus. The disease made its appearance from margin or apex and proceeded towards base of the leaflets. The spots were light-brown in colour and demarcated often by concentric zones. In severe cases numerous minute black dots were also developed throughout the infected regions. Isolations from such diseased fragments consistently yielded the conidia of *Alternaria tenuis* Nees. After about a month dark brown perithecia of an ascomycetous fungus were also observed in the same culture tubes which on examination revealed as *Pleospora infectoria* Luckel.

In order to establish the relationship in these two stages mono-conidial and mono-perithecial cultures were raised separately. After about a month the perithecia of *P. infectoria* were observed in the mono-conidial cultures of *A. tenuis*. Similarly the mono-perithecial cultures resulted both conidial as well as perithecial stages.

From perusal of the literature it revealed that neither *A. tenuis* nor its perfect stage is reported on the leaves of this host.

The author is grateful to Prof. S. S. Prasad for his valuable help and encouragement.

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Muzaffarpur, February 15, 1974.

#### EFFECT OF PANACIDE ON SOME GREEN AND BLUE-GREEN ALGAE

ALTHOUGH algae help to maintain a balanced aquatic eco-environment, they can pose problems in garden tanks, ponds, lakes, etc. Chemical control of algae has been stressed by many workers<sup>1-3</sup>. However, biocides have to be carefully evaluated before recommending them for control or eradication of algae.

Panacide (BDH) (Dichlorophen), a well known algicide, has been screened against five species of green and blue-green algae. Unialgal cultures (grown in Gerloff's modification of Chu No. 10 solution<sup>4</sup> under continuous fluorescent illumination and at a temperature of  $28^{\circ} \pm 1^{\circ} \text{C}$ ) of *Scenedesmus obliquus*, *Chlorella pyrenoidosa*, *Myxosarcina spectabilis*, *Aulosira prolifica* and *Nostoc* sp. (which frequently occur here in garden tanks, and lily ponds) have been used in the tests. In these tests, thirteen concentrations of Panacide, ranging from 1 ppm to 80 ppm were used. The chemical was added to the cultures containing approximately 3.5

million cells per ml after 10 days of inoculation. The presence of algal growth was determined visually as well as microscopically at 5-day intervals upto 30 days, and the amount of growth in each treated flask was compared to that in the control. The effect of Panacide was determined by counting the percentage number of disintegrated and bleached cells. The lowest concentration necessary to kill 100% of the cells and the failure of the treated cultures to exhibit any growth upon subculturing in fresh medium was considered to be the minimum lethal dose.

Panacide proved to be toxic to all the five test algae. When the biocide was present at more than 20 ppm the growth of *Scenedesmus*, *Chlorella*, *Myxosarcina* and *Aulosira* was strongly inhibited, and degeneration of the cells set in within seven days of the treatment. The younger populations of *Scenedesmus* and *Chlorella* were more sensitive to the chemical than older ones. *Nostoc* was even more sensitive and 100% cells could be killed by using a concentration of only 10 ppm. The toxic effect became intensified with incubation time upto 20 days, but not thereafter. The observed damage to the cells was irreversible, and the chemical could not be leached out even after repeated subculturing in fresh media.

Panacide is a good candidate for the control of common noxious and resistant algae.

The authors are grateful to BDH Chemicals, Ltd., England, for providing the sample of Panacide for screening tests.

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# **OSTRINIA KASMIRICA MOORE, A NEW HOST OF SERRATIA MARCESCENS BIZIO FROM INDIA**

THE lepidopterous tissue-borer, *Ostrinia kasmirica* Moore, feeds on the thistle plant, *Cnicus arvensis* (Family Compositae), in the Kulu valley (Himachal Pradesh). With a view to study its life-history,

the field-collected larvae were reared on cut-pieces of thistle stem, brought from the Kulu valley, in the laboratory (18-8°-24-9° C) at Ludhiana. Most of the hibernating larvae were observed dying of a bacterial infection. The causative organism was identified as two non-chromogenic strains of *Serratia marcescens* Bizio. The infected larvae exhibited sluggishness in crawling, anal watery discharges and shrivelling of bodies. The moribund larvae were soft to touch, brownish-black and turned jet-black after death. The microscopic examination of the anal discharges revealed the presence of small gram-negative rods.

The larvae of *Ostrinia nubilalis* (Hübner), a close relative of *O. kasmirica* and a serious pest on corn, have been reported to die of *Serratia* infections by Raun and Brooks (1963) in Iowa, U.S.A. *Serratia marcescens* has been earlier found to cause disease among the larvae of *Agrotis ypsilon* Rott. (Chattopadhyay and Mukherjee, 1955), *Nephantis serinopa* Meyrick (Antony and Kurien, 1961) and *Azygophleps scalaris* (Rangaswami *et al.*, 1970) under field conditions in India. The cultures of *Athalia proxima* Klüg (Bogawat *et al.*, 1966) and *Prodenia litura* (Fabricius) (Pandey and Rangarajan, 1967) were also observed to suffer heavy mortality due to this bacterium in the laboratory. The present report of mortality among the field-collected hibernating larvae of *O. kasmirica* owing to *S. marcescens* is the first record from India.

The research work was financed under PL-480 Project A7-Ent-43 by the Agricultural Research Service of the U.S. Department of Agriculture. The authors are grateful to Dr. Yoshinori Tanada and Mr. Gerard M. Thomas of the University of California, U.S.A., for identification of the pathogen.

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## SHORT SCIENTIFIC NOTES

### A Technique for Separating the Early Blastomeres of *Limnaea*

Useful methods such as polyacrylamide gel electrophoresis are now widely applied for investigating problems of molecular embryology. Following the techniques of Edstrom one can now analyse the protein (and RNA) of single cells such as erythrocytes<sup>1</sup>. For such and other interesting work in the field of molecular embryology it would be first necessary to isolate the blastomeres of early morula. In this note we describe a simple method for isolating, *i.e.*, physically separating the early blastomeres of *Limnaea*, a common pond snail.

This technique is based on the earlier findings on the action of mercaptoethanol on *Limnaea* blastomeres<sup>2</sup>, namely, that this chemical decreases the adhesive property of blastomeres although complete separation is difficult to obtain. The following operational techniques have enabled us to separate the blastomeres in a comparatively simple way.

The freshly laid egg mass, removed from below the surface of floating leaves kept in the laboratory aquarium (or in the pond), was rolled on a piece of Whatman filter-paper No. 1 with the help of a needle. The eggs (*i.e.*, inside the capsule but now freed from the jelly) were picked up with the help of a camel hair brush. Under the dissecting microscope operation with needles can remove further traces of the jelly mass. (These liberated eggs dry up quickly.)

If the eggs are now in 2- or 4-cell stage, the capsule is punctured with the help of needles under a dissecting type microscope. The operation is simple if the jelly has been mostly removed. The capsule fluid dries up quickly and so a solution or medium is required for maintaining the eggs therein. Jockusch<sup>3</sup> described an inorganic salt solution (henceforth referred to in this note as Jockusch medium). A variant of this is Ca-free Jockusch medium, *i.e.*, omitting the ca-salt from the normal composition of Jockusch medium.

0.1 M mercaptoethanol (in Ca-free Jockusch) was used to loosen the blastomeres. In view of the toxicity of this medium it is advisable to further dilute it 5 times (with Ca-free Jockusch). After puncturing and tearing away of the capsule kept in this medium, a further physical operation is necessary. Small hair loops were prepared with the help of comparatively soft hair from arms or legs. These were fixed into long thin glass tubes by melting the tip of the tube. Under the dissecting type micro-

scope one can gently press the loop in the middle of the cleaving cell. Gentle operation with the other loop can quickly physically separate the two blastomeres. This is apparently not possible unless the adhesive property is made to diminish with the help of mercaptoethanol in Ca-free Jockusch. After separating the blastomeres, it is advisable to slowly pipette out this medium and replace it with normal Jockusch medium. The separated blastomeres from 2- or 4-cell stages continue to divide in this medium and the expected mosaic type of cleavage has been observed.

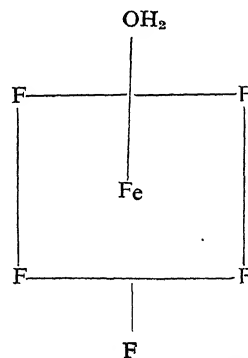
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### A Mossbauer Study of Dipotassium Aquo-Pentafluoroferrate (III)

A single crystal X-ray structure determination of  $K_2[FeF_5(H_2O)]$  has been reported recently<sup>1</sup>. The structure consists of four fluorine atoms in the square plane and the other fluorine atom and water molecule occupying the trans positions. The coordinated oxygen atom is farther from the iron atom ( $Fe-O = 2.07 \text{ \AA}$ ) than the fluorine atoms ( $Fe-F = 1.92 \text{ \AA}$ ). The anions are linked into chains by strong hydrogen bonding ( $O \cdots F = 2.54 \text{ \AA}$ ).



Structure of  $[FeF_5(H_2O)]^{2-}$  anion.

We have studied this compound with Mossbauer spectroscopy in order to know the effect of replacement of one fluorine atom in  $K_3[FeF_6]$  by a water molecule.

The complex  $K_2[FeF_5(H_2O)]$  was prepared according to the method of Palmer<sup>2</sup>. Anal. Calcd. for  $K_2[FeF_5(H_2O)]$ , Fe, 22.61%. Found: Fe, 22.32%. The Mossbauer spectrometer used was a constant velocity-drive type. The source used was  $^{57}Co$  in Cu. The Mossbauer spectrum of  $K_2[FeF_5(H_2O)]$  was recorded at liquid nitrogen temperature (77° K).

The Mossbauer spectrum of  $K_2[FeF_5(H_2O)]$  consists of two distinct lines as a result of quadrupole splitting. The value of the quadrupole splitting ( $\Delta E_Q = 0.60 \pm 0.05$  mm/sec), and the isomer shift ( $\delta = 0.20 \pm 0.05$  mm/sec) are consistent with the formulation of the complex as one containing iron(III) in the  $S = 5/2$  spin state. It is interesting to note that the Mossbauer spectrum of  $K_3[FeF_6]$  and  $K_3[Fe(CN)_6]$  exhibit single line spectra whereas the replacement of one F or CN by  $H_2O$  leads to the appearance of quadrupole splitting due to the changes in the environment and symmetry.

Infrared spectrum of  $K_2[FeF_5(H_2O)]$  was recorded in KBr pellets on a Perkin Elmer Model 521 instrument and the infrared spectrum of the compound exhibits a medium intense band at  $3100\text{ cm}^{-1}$  due to the coordinated water<sup>3</sup>.

The author is indebted to the UGC and the faculty research fund of the University of Bombay for support of this research.

Inorganic and Physical A. SYAMAL.

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#### A Method to Increase Protein Content in Rice

Rice (*Oryza sativa* L.) is a low protein cereal. Attempts are being made to evolve protein-rich high yielding rice through breeding so that people living mostly on rice can have a substantial increase in the total protein intake in their diets.

Das Gupta and Bera<sup>1</sup> explored the possibility of increasing the efficiency of the high yielding rice varieties to utilize the nitrogen applied to the soil by the application of the trace element molybdenum and noted that the element significantly stimulated the utilization of nitrogen resulting in increased growth and yield. The protein content of the grain was also substantially increased by molybdenum<sup>2</sup>. Although by nitrogen fertilization alone, the protein content could be increased, a further increase could be obtained by molybdenum.

In view of the quantitative increase in the protein content in the rice grain it was considered necessary to examine how far the quality of protein is affected by molybdenum. The protein-bound amino acids in the grain (of cv. 'IR 8' grown in different combination of nitrogen and molybdenum) were therefore studied chromatographically and their concentrations in the proteins estimated colorimetrically following the techniques described by Das Choudhury *et al.*<sup>3</sup>.

TABLE I

Effect of nitrogen and molybdenum on protein bound amino acids (mg per g dry weight) in the grains of rice (Var. IR 8)

Amino acids	N <sub>0</sub>		N <sub>1</sub>		S.E.m.
	M <sub>0</sub>	M <sub>1</sub>	M <sub>0</sub>	M <sub>1</sub>	
Leucine	.. 6.7	8.0	11.2	13.4	± 1.5
Phenylalanine	.. 2.3	3.5	3.2	3.7	± 0.3
Methionine and valine	5.5	8.5	6.3	5.8	± 0.7
γ-Amino butyric acid	—	—	—	—	—
β-Alanine	.. +	2.0	1.6	3.1	± 0.4
Alanine	.. 7.5	10.0	11.0	13.3	± 1.2
Threonine	.. 2.2	3.5	3.7	5.5	± 0.6
Serine and glycine	.. 7.5	9.5	6.4	8.1	± 0.6
Glutamic acid	.. 1.7	3.5	12.1	16.9	± 3.6
Aspartic acid	.. 8.5	9.5	14.2	18.7	± 2.3
Lysine	.. 4.2	4.5	4.5	6.7	± 0.6
Arginine and histidine	3.5	5.0	4.5	+	± 0.4
Proline	.. +	+	+	2.7	—
Asparagine	.. —	—	—	—	—
TOTAL	.. 46.0	67.5	78.7	97.9	± 10.8

N<sub>0</sub>=No nitrogen, N<sub>1</sub>=Nitrogen applied @ 400 kg/ha, M<sub>0</sub>=No molybdenum, M<sub>1</sub>=Molybdenum applied @ 40 g/ha; +=Trace.

The data presented in Table I clearly show that most of the essential amino acids are present in higher concentrations in the proteins in presence of nitrogen and molybdenum. The addition of



molybdenum increases the total protein-bound amino acid content by about 2% over nitrogen. The substantial increase in the concentrations of the essential amino acids including lysine, serine and glycine, glutamic acid and others in the grain proteins suggests the great potentiality of molybdenum in the culture of protein-rich rice varieties.

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### Colchicine Induced Mixoploid in Coriander

Coriander (*Coriandrum sativum*) is one of the important condiments being used in cooking, flavouring beverages and in medicine. Increase in oil content and green foliage following polyploidy has been reported in this crop<sup>1</sup>. The present study was undertaken to induce polyploidy by treating seedlings at cotyledonary leaf stage with 0.15 and 0.25% of colchicine in aqueous solution for 24 hours. After treatment the seedlings were thoroughly washed with distilled water.

40 days after treatment a mixoploid plant with a few leaves which were more dark, broad and leathery in consistency was observed among the plants treated with 0.25% colchicine. The flower buds from different umbels were fixed separately in acetic alcohol (1:3) and PMC smears were made using 1% acetocarmine. In two umbels the chromosome number was  $2n=44$  as against  $2n=22$  in the normal ones. The mean association of chromosomes in the tetraploid cells was  $2.40\text{ IV} + 1.34\text{ III} + 14.60\text{ II} + 1.20\text{ I}$ , the range being  $1\text{ IV} + 1\text{ III} + 18\text{ II} + 1\text{ I}$  to  $5\text{ IV} + 1\text{ III} + 10\text{ II} + 1\text{ I}$  per PMC at metaphase 1.

Meiotic irregularities in the form of laggards and bridges were observed during first anaphase in 20% and in 1.25% cells, respectively.

The study of the mixoploid plant is interesting in view of the low frequency of quadrivalents observed in the tetraploid cells. In an autotetraploid large number of quadrivalents is expected, since each chromosome is present in quadruplicate. But in the present study the mean frequency of quadrivalents observed in tetraploid cells was 2.40 which is rather low. The low quadrivalent frequency may be due to lack of perfect homologous partners<sup>2</sup>. If

this is true, then it is quite likely that *Coriandrum sativum* which itself is a monotypic species may be of polyploid origin with two closely related genomes since the possibility of only quadrivalents at octoploid level has been reported in this crop<sup>2</sup>. Such a possibility has been recently reported in a strict diploid species like mung bean (*Vigna radiata*)<sup>3</sup>. Thorough investigations are required to explain the nature and causes of low quadrivalent frequency at tetraploid level. This may throw light on the phylogenetic position of this crop. Studies in this direction are under progress.

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### Collar Rot of Sunflower (*H. annuus*) A New Host Record from India

Collar rot of sunflower caused by *Sclerotium rolfsii* (Sacc.) was first observed in the month of September 1972 at Oil Seed Research Station, Latur, in Maharashtra State. A review of literature shows that the fungus has not been reported so far on sunflower from India, although it has been reported on sunflower from Tucuman province<sup>1</sup>, Argentina<sup>2</sup>, Queensland<sup>3</sup> and Uruguay<sup>4</sup>. Affected plants in the field were recognised by sudden wilting and drying. Collar portion of the plant was the general point of attack, on which a tuft of white mycelium was found growing. Infection was mostly in seedling stage. Later on brown sclerotia were produced on the affected portion of the plant. Pathogenicity of the fungus was established by soil inoculation method. The inoculum was prepared by growing the fungus on sterilized crushed maize seed medium. Typical symptoms were produced within a week of inoculation which were identical to those produced in the field.

Division of Plant Pathology, V. V. DATAR.  
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## REVIEWS AND NOTICES OF BOOKS

**Understanding the Earth.** Edited by I. G. Gass, P. J. Smith and R. C. L. Wilson, at the Open University. ELBS Edition. (The English Language Book Society, The Artennis Press Ltd., Sussex. MCM LXXIII), 1972, pp. 383. Price £1.40.

Many questions regarding the earth has been baffling to the human brain: the origin of this planet, its location and relation to other celestial bodies, the evolution of both living and non-living objects of the planet, the composition and structure of the earth, the dynamic properties of earth like the formation of the continents, oceans, mountains, the atmosphere and so on. Probing into earth's history has always been difficult, since the earth continues to be dynamic and the observations are limited in time, whereas the changes on earth are spread over a period of about 5,000 million years. As such, there are observations and speculations pertaining to earth, accumulated through centuries on various aspects of its past history. However, there is a vast improvement in the understanding of what is going on the earth's surface, thanks to the advances made in Geology and Space Science. Earth Science thus developed as a branch has very wide scope and to place all the aspects in one volume is a difficult and stupendous task. This is the reason why there is no single text book in this field of Science.

*Understanding the Earth* edited by Gass, Smith and Wilson is formulated as an inexpensive text in Earth Science as a part of the Foundation Course in Science. It includes twenty-seven articles contributed by leading scientists. Some of the articles, within the limit and scope, are mentally stimulating and superb, particularly to mention are: The minerals and rocks, measuring geological time, dealing with the radiometric dating of rocks, the composition of earth, the earth-moon system, looking back through time, continental drift, and plate tectonics. A few other articles are hastily written and are incomplete. The place of some chapters, though extremely interesting, like the one on 'Mohole Project' and 'The Environmental Science' may not be in a book like this. The chapters like 'The Oxygen Cycle' could have been well planned to include the cyclic exchange processes of a number of other elements. The editors claim the up-to-date nature of the book by reproducing a 1970 article of the *Scientific American*! Would it not have been nice to have a single chapter on magnetic characteristics

of earth, incorporating the contents of the articles in 4, 17 and 18? The chapters dealing with continental drift, and sea-floor spreading should have been immediately following the chapters on 'Plate Tectonics' and 'Orogeny'.

However this inexpensive text on "Understanding the Earth" on the whole is very informative and useful not only to the students of Geology but also to the students and teachers of Science in general.

G. V. ANANTHA IYER.

**Ecology and Biogeography in India.** Edited by M. S. Mani. (Dr. W. Junk b.v. Publishers, The Hague), 1974. Pp. xix+773, 163 figs. and 75 photographs, 2 folding maps and 58 tables. *Series Monographiae Biologicae*, Volume 23. Price Dutch Guilders 190.

I would like to begin by expressing my great admiration for Prof. Mani, who has produced yet another *magnum opus* after his significant work on high altitude insects; this time on biogeography of India. India is a particularly fascinating region biogeographically, lying as it does at the cross-roads for the dispersal of the great diversity of living organisms produced in the evolutionary theatre of the old world tropics. This great flora and fauna is being rapidly decimated and there is a great urgency of learning as much as possible about it before its distribution patterns become totally obscured by large-scale extinctions. The present volume makes an important contribution towards this. It includes twenty-four papers, eleven of them by Prof. Mani himself, and the other thirteen by a number of specialists on the physiography, geological history, climate, flora and fauna of the Indian region. This manages to cover the physical background and the vegetation quite thoroughly, and the insects and vertebrates fairly adequately. Inevitably, a few of lacunae remain. Thus, the account of bird distribution appears to be largely based on Blanford's nineteenth century work, and does not incorporate Salim Ali's and S. Dillon Ripley's major contributions. There are also a number of minor errors, such as the confusion between *Garrulus* and *Garrulax* on page 676. Some such errors are, however, inescapable in a work of this proportion and do not really detract from the value of the account.

The detailed descriptive accounts of the first twenty-three chapters culminate in Prof. Mani's essay on the biogeographical evolution in India.

His major novel thesis is that the peninsular India was the theatre of the evolution of the greatest bulk of Indian flora and fauna nearly upto the Pleistocene times. Incursions of Indo-Chinese, Malayan and Palaearctic elements are relatively recent, although they have had a tremendous impact. Much of this thesis is put forth in a manner thoroughly convincing, at least to a non-specialist like me. What does bother me in this account though is the very explicit interpretation of evolution in terms of life cycles of taxa. For example, Prof. Mani talks of the death of vast populations of dominant groups, perhaps from faunal senility and exhaustion. Helen Spurway has termed such an approach Vaishnavite, and therefore it is perhaps not surprising that Prof. Mani goes on to talk of completely disjunct populations evolving into 'identical' forms like so many Krishnas simultaneously appearing in an identical form with each of his innumerable wives. Some of these interpretations are thoroughly in conflict with modern day Saivite evolutionary theory, and will no doubt meet with serious resistance.

Such disagreements apart I thoroughly recommend this volume to all those interested in biogeography and find it most gratifying that such a significant work has emerged from this biogeographically fascinating continent. M. G.

#### Immunopathology: Methods and Techniques.

Editors: Theodore P. Zacharia and Sydney Breese, Jr. (Marcel Dekker, Inc., New York), November 13, 1973. Pp. 261. Price \$19.75.

The present volume, the second in 'Immunology Series', deals with basic and applied aspects of immunology.

The chapter on "The problem of the immunogen receptor on the thymocyte plasma membrane" details various techniques used for detection of immunogen receptor on cell surface and presents the author's data on detection of cell surface immunoglobulin on thymic and splenic lymphocytes by membrane solubilisation and sodium dodecyl sulfate acrylamide gel electrophoresis of immunoprecipitates.

In the chapter on "Cellular mechanism for the induction and maintenance of immunological

tolerance" the authors distinguish between two types of immunotolerance; the central wherein there is specific suppression of antibody forming mechanism which can be demonstrated by transfer of lymphoid cells to immunologically neutral environment where the unresponsiveness is retained; and peripheral where capacity to produce an immune response is impaired either due to presence of large dose of antigen or elaboration of tolerogenic T-cell or B-cell products.

The article 'Antibody-macrophage interaction and the immune response' mentions that antigens are handled differently in non-immune and immune animals. In non-immune animals the antigen processed by macrophage produces an immunogenic RNA which in turn stimulates an immunocompetent cell, whereas in immune animals antibody directs the antigen towards a population of non-processing macrophages which destroy the antigenicity of the antigen or activate the macrophage enzymes which degrade the antigen. However the mechanisms proposed are conjectural. Details of methods for assay, of reaginic antibody and mediators can be found in the article 'Molecular mechanism of the human allergic response'.

Amongst the other informative articles, 'Immunological renal disease' describes the pathophysiological mechanisms of this autoimmune disease; Immunoelectron microscopic applications of ferritin-tagging' details the application of this technique for study of viruses, bacteria, parasites and cell surface; and 'Acquired immunity in Malaria' outlines the methodology required for studies in the field of malaria immunology and the current state of knowledge in this field.

This volume summarises and integrates two broad areas: Immunological reactions at the cellular level and immunological phenomenon at the clinical level.

R. NAYAK.

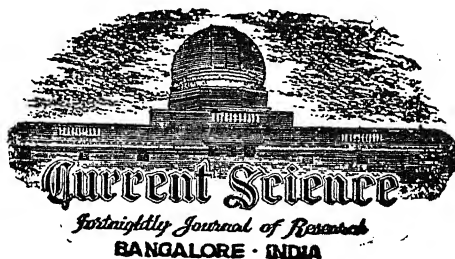
#### Books Received

*Physics—With Illustrative Examples from Medicine and Biology* (Vol. 1—*Mechanics*). By G. B. Benedek and F. M. H. Villars. (Addison-Wesley Pub. Co., Inc., Reading, Massachusetts 01867), 1973. Pp. 684. Price not given.

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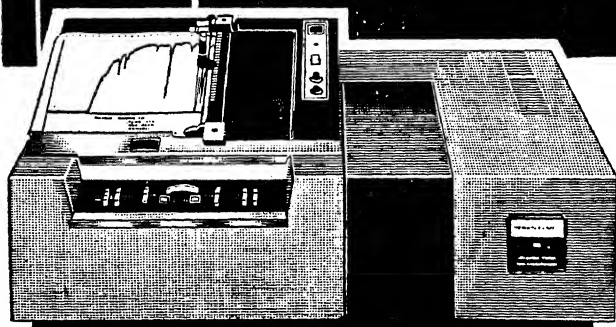
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# EFFECT OF ANTIBIOTICS AND ANTIMETABOLITES ON THE INDUCTION OF L-ARABINOSE ISOMERASE IN *SALMONELLA TYPHIMURIUM*

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## ABSTRACT

The effect of antibiotics (mitomycin C, actinomycin D and rifampin) and the antimetabolite, cycloheximide, on the induction of L-arabinose isomerase in *Salmonella typhimurium* has been studied. Cycloheximide, at a concentration of 10 µg/ml which does not interfere with the normal protein synthesis, during the period of study, interferes with the induction of the enzyme. This is due to the synthesis of faulty mRNA. Under the same conditions, there is no effect on the overall rate of protein synthesis. Mitomycin C, actinomycin D and rifampin inhibit the induction of the isomerase. As expected, actinomycin D and rifampin inhibit the rate of synthesis of L-arabinose isomerase. The results indicate that the induction of L-arabinose isomerase is controlled at the level of transcription.

## INTRODUCTION

THE studies on the induction and repression of enzymes in bacteria have helped to a great extent to understand the genetic control of the gene activity. Most of them were first carried out with the help of operon in *E. coli* and *S. typhimurium* cells. These studies led to a revision of the well known concept of Jacob and Monod, which turned out to be more or less correct in great details. Considerable genetic investigations have been carried out with the *ara* operon in *E. coli* & *S. typhimurium*. Repression of the induction of L-arabinose isomerase in *Salmonella typhimurium* and its reversal by cyclic 1', 5'-AMP have already been reported from this laboratory (1). We have also studied the effect of L-arabinose on the induction.

The antibiotics and antimetabolites which block some specific steps of protein and RNA synthesis have been widely used to understand the mechanism of genetic control of protein synthesis. It was therefore of interest to study the effects of these antibiotics and antimetabolites on the induction of L-arabinose isomerase. In the present investigation, fluorouracil, actinomycin D, mitomycin C and rifampin have been used to show that the *ara* enzyme synthesis is regulated at the level of transcription.

## MATERIALS AND METHODS

**Bacterial Strain.** *S. typhimurium* LT2 was kindly supplied by Prof. Myron Levine of the University of Michigan, Ann Arbor, Michigan, U.S.A.

**Chemicals.** Fluorouracil was a gift from Prof. S. P. Chakraverty of the Institute of Microbiology,

Rutgers State University, Brunswick, New Jersey, U.S.A. Mitomycin C was kindly supplied by Prof. Y. Takagi of the Kyushu University, Fukuoka, Japan. Rifampin was a gift from Dr. J. Gelzer, Director, Microbiology Division, CIBA Pharmaceutical Company, New Jersey, U.S.A. Actinomycin D was obtained from Merck, Sharp and Dohme Research Laboratories, New Jersey, U.S.A. L-Arabinose was obtained from Calbiochem, Los Angeles, U.S.A. All other chemicals were commercial preparations and were of analytical grade.

**Growth medium.** Growth of *S. typhimurium*, Induction of L-arabinose isomerase, Assay of L-arabinose isomerase and EDTA treatment of cells for induction experiments have been described elsewhere (2).

**Measurement of the overall rate of protein synthesis.** The overall rate of protein synthesis was measured by following the incorporation of  $^{14}\text{C}$ -L-phenylalanine into *S. typhimurium*. Cells were grown from an overnight grown inoculum as described before (3). When the cell suspension reached an absorbance of 0.12 ( $1.6 \times 10^8$  cells/ml), 16 µmoles of L-arabinose and 0.05 µmole of  $^{14}\text{C}$ -L-phenylalanine (having  $5 \times 10^4$  counts/min) were added per ml of the cell suspension. At 30th minute following the addition of  $^{14}\text{C}$ -L-phenylalanine, fluorouracil was added at a final concentration of 10 µg/ml. At different time intervals, aliquots (1 ml) were collected in pre-chilled tubes containing 1 ml of 10% trichloroacetic acid. The mixtures were kept in ice for 30 min and the trichloroacetic acid insoluble precipitates were collected by filtration through millipore filters. Each filter was washed with 25 ml of cold 5% trichloroacetic acid, mounted on planchet, dried and counted in a windowless gas flow counter of Bhabha Atomic Research Centre, Trombay, India.

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## RESULTS

**Effect of fluorouracil on the induction.**—Fluorouracil is known to be incorporated into RNA in place of uracil and thus to produce defective mRNAs. This pyrimidine analogue at a concentration of 10  $\mu\text{g/ml}$  had little effect on the growth of *S. typhimurium* up to about 45 min following its addition; at which time growth is inhibited, and finally the growth completely halts after about 75 min (results not presented). Fluorouracil (10  $\mu\text{g/ml}$ ) was added 30 min following the addition of the inducer and its effect on the enzyme induction was studied for another 40 min (Fig. 1). The induction of L-arabinose isomerase was inhibited immediately after the addition of fluorouracil.

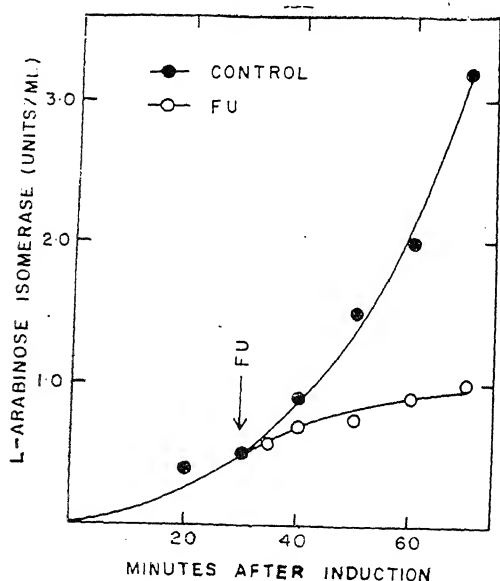


FIG. 1. Effect of fluorouracil on the induction of L-arabinose isomerase. Samples were removed at the indicated time intervals for the assay of the enzyme. Fluorouracil (10  $\mu\text{g/ml}$ ) was added at 30th minute following the addition of the inducer.

As discussed already, fluorouracil is incorporated in place of uracil and thus produces defective mRNA. This may lead to cessation of protein synthesis or the protein synthesised from the faulty mRNA may not be enzymatically active. In the first case the overall rate of protein synthesis will be affected in presence of fluorouracil whereas in the second case fluorouracil will have no effect. It is clear from results presented in Fig. 2 that fluorouracil had little effect on the overall rate of protein synthesis at least for 40 min, the period for which its effect on the induction of L-arabinose isomerase was studied.

**Effect of mitomycin C on the induction of L-arabinose isomerase.**—Mitomycin C is known to interfere with the duplication of DNA but does not normally inhibit RNA and protein synthesis<sup>9</sup>. As expected, the antibiotic (5  $\mu\text{g/ml}$ ) had no effect on the induction process. The level of the enzyme increased up to 30 min but after that there was no increase of enzyme level (Fig. 3). The action of the antibiotic was in parallel with its effect on the growth of *S. typhimurium*. In presence of 5  $\mu\text{g/ml}$  of mitomycin C the growth continued at the normal rate for about 30 min and then completely ceased (results not presented).

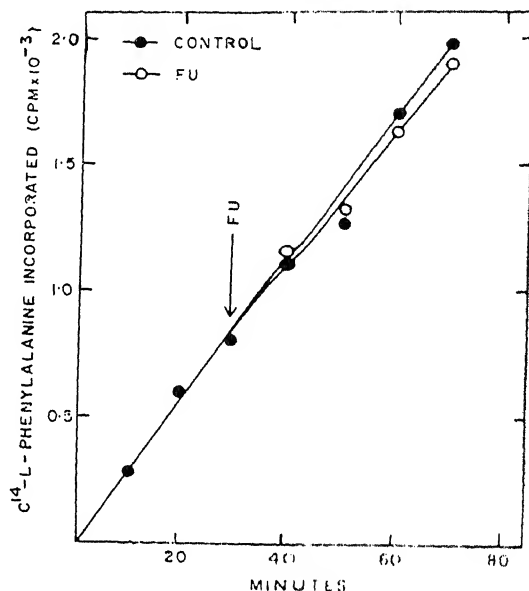


FIG. 2. Effect of fluorouracil on the overall rate of protein synthesis. The rate of protein synthesis was measured as described under 'Materials and Methods'. Fluorouracil (10  $\mu\text{g/ml}$ ) was added at 30th minute following the addition of L-arabinose and C<sup>14</sup>-L-phenylalanine.

The lack of interference by mitomycin C on enzyme induction for 30 min may be due to the weak permeability of the compound in *S. typhimurium*. This was tested by studying the effect of the antibiotic on the induction of L-arabinose isomerase in EDTA-treated cells. Results presented in Fig. 4 clearly indicate that in EDTA-treated cells a longer period (60 min; compare Figs. 3 and 4a) is required for mitomycin C to exert its effect on the induction of the isomerase. The kinetics of enzyme induction in presence of mitomycin C run parallel with that of the growth rate (Figs. 4a and b). The growth rate in cells treated with EDTA is slower than that of non-treated cells. Therefore, the delay in stopping the induced syn-

thesis of L-arabinose isomerase is not due to the permeability of *S. typhimurium* cells to mitomycin C.

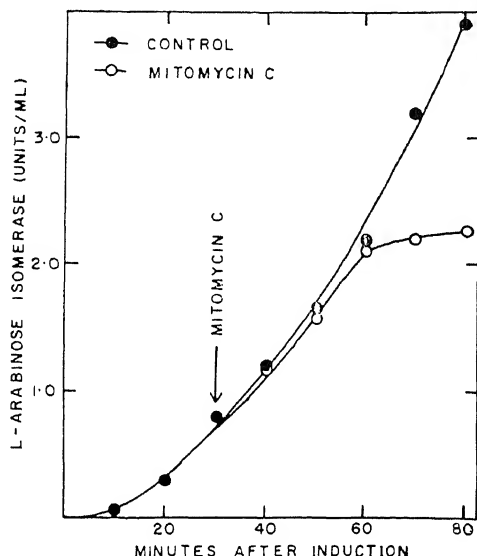


FIG. 3. Effect of mitomycin C on the induction of L-arabinose isomerase. Mitomycin C (5  $\mu$ g/ml) was added at 30th minute following the addition of the inducer.

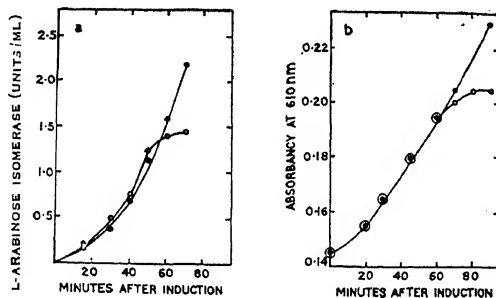


FIG. 4. Effect of mitomycin C on the induction of L-arabinose isomerase (a) and growth of *S. typhimurium* (b) in EDTA-treated cells. The EDTA-treatment of the cells was done as described earlier<sup>5</sup>. Mitomycin C (5  $\mu$ g/ml) was added along with the inducer. —●—, control; —○—, mitomycin C.

**Effect of actinomycin D on the induction.**—Actinomycin D is a potent inhibitor of DNA-dependent RNA synthesis<sup>10-13</sup>. Being a Gram-negative organism *S. typhimurium*, however, was insensitive to the action of actinomycin D. The cells could, however, be made sensitive to the action of actinomycin D by treatment with EDTA<sup>14</sup>. The effect of actinomycin D (20  $\mu$ g/ml) on the induction of L-arabinose isomerase was studied for 70 min both in untreated and EDTA-treated cells.

The results are presented in Table I. As expected, actinomycin D had no effect on the induction in untreated *S. typhimurium*. The induction in EDTA-treated cells was, however, inhibited to an extent of 60% by actinomycin D.

TABLE I

Effect of actinomycin D on the induction of L-arabinose isomerase in normal and EDTA-treated cells

Cells	Actinomycin D	
	—	+
Untreated	(Units/ml)	
EDTA-treated	3.1	3.0
	2.3	0.9

Actinomycin D (20  $\mu$ g/ml) was added along with the inducer and the induction was carried out for 70 min.

**Effect of rifampin on the induction.**—Rifampin is a derivative of rifamycin which is known to interfere with the RNA synthesis by binding directly with DNA-dependent RNA polymerase<sup>15,16</sup>. Therefore it was expected that this antibiotic would interfere with the enzyme induction. The effect of varying concentration of rifampin on the induction was studied first (Fig. 5). At a concentration of 20  $\mu$ g/ml of rifampin, the enzyme production was inhibited to an extent of 83%. A comparatively high concentration of rifampin (50  $\mu$ g/ml) was chosen to study the time course of its action (Fig. 6). Rifampin completely blocked the enzyme induction immediately following its addition.

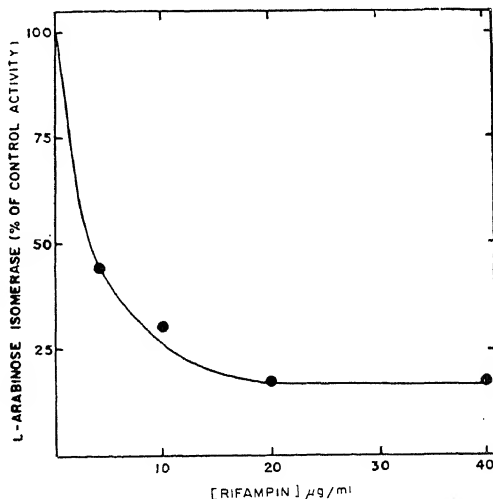


FIG. 5. Effect of varying concentration of rifampin on the induction of L-arabinose isomerase. Induction was carried out for 70 min. Varying concentrations of rifampin as indicated was included along with the inducer in the medium. Control activity (100 per cent) denotes the amount of enzyme induced in the absence of rifampin.



## DISCUSSION

L-Arabinose induces the enzyme L-arabinose isomerase in *S. typhimurium* growing in MM. having glycerol as the carbon source. The basic features of the process of induction of L-arabinose isomerase are the same as that of the induction of  $\beta$ -galactosidase<sup>5</sup>. However, in certain aspects, specially with reference to both positive and negative control exerted by the inducer, the L-arabinose system seems to be somewhat different from  $\beta$ -galactosidase system<sup>3,4</sup>. Though it seems from the recombination studies that the control involves the regulation of synthesis of *ara* mRNA<sup>17</sup>, no direct evidence for this has yet been found. An attempt has been made in the present investigation to find out the level at which this control is exerted in the *ara* operon.

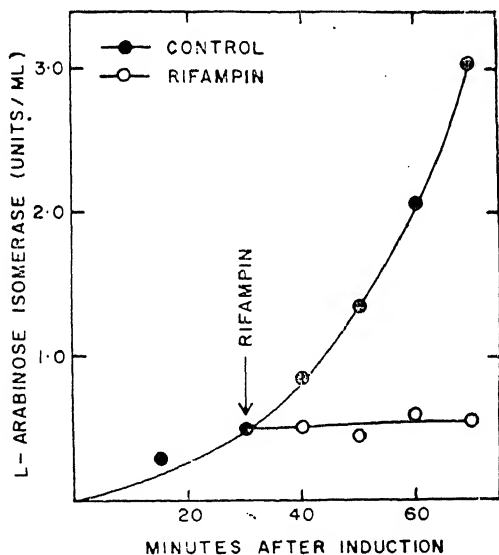


Fig. 6. Effect of rifampin on the kinetics of induction of L-arabinose isomerase. Rifampin (50  $\mu$ g/ml) was added at 30th minute following the addition of the inducer.

The induction of the enzyme is stopped in presence of the antimetabolite fluorouracil (Fig. 1). Even at a concentration (10  $\mu$ g/ml) which does not interfere with the rate of growth of the microorganism during the period of study (results not presented), very little active enzyme is synthesised. The failure to produce active enzyme in presence of fluorouracil is most probably not due to lack of transcription or translation, but is the result of faulty translation because the rate of overall protein synthesis as measured by  $C^{14}$ -L-phenylalanine incorporation is not affected in presence of fluorouracil (Fig. 2). The effect of fluorouracil

on the synthesis of L-arabinose isomerase in *S. typhimurium* resembles its effect on  $\beta$ -galactosidase synthesis<sup>18-20</sup>. However, it differs from that on L-arabinose isomerase synthesis in *L. plantarum* as reported earlier from this laboratory<sup>21</sup>. In *L. plantarum* fluorouracil has no effect on L-arabinose isomerase synthesis.

Mitomycin C (5  $\mu$ g/ml) which interferes with the duplication of DNA does not interfere with the induction of the enzyme up to 30 min (Fig. 3) following its addition. After 30 min the growth of the microorganism is stopped (results not presented), hence there is no further synthesis of the enzyme. Even when *S. typhimurium* cells were treated with EDTA to increase their permeability to mitomycin C, the effect of the antibiotic on the induction of L-arabinose isomerase is in parallel with its effect on the growth of the microorganism (Fig. 4). The nongrowing condition of the cells is responsible for no further synthesis of the enzyme. The results obtained with *S. typhimurium* are in conformity with those reported in case of the same enzyme from *P. pentosaceus* and *L. plantarum*<sup>21,22</sup>. It should be mentioned here that penicillinase induction in *S. aureus*<sup>23</sup> and  $\beta$ -galactosidase induction in *E. coli*<sup>24,25</sup> cannot take place if the cells are treated with mitomycin C.

The antibiotic actinomycin D is widely used to study the direct involvement of mRNA. Being a Gram-negative organism *S. typhimurium* is not sensitive to actinomycin D. However, in EDTA-treated cells the induction of enzyme is inhibited in presence of actinomycin D (Table I). The slight difference in the levels of the enzyme in untreated and treated cells (in absence of actinomycin D) is a reflection of the difference in the growth rate in the two cases.

As expected, rifampin completely blocks the enzyme induction (Fig. 6). The induction of the enzyme decreases exponentially with the increase in the concentration of rifampin (Fig. 5). In presence of 20  $\mu$ g/ml of the antibiotic, the level of L-arabinose isomerase is only 17% of that in absence of antibiotic. With further increase in the concentration there is no more decrease in the level of the induced enzyme.

The results presented in this paper clearly show that as in case of most inducible enzymes, L-arabinose isomerase induction also is regulated at the level of transcription.

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# ESTABLISHMENT OF SYMBIOSIS IN VITRO, BETWEEN RHIZOBIUM AND PEA (*PISUM SATIVUM*) ROOT CALLUS

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## ABSTRACT

Pea (*Pisum sativum*) root callus was inoculated with *Rhizobium leguminosarum* and studied for the establishment of symbiosis. Infection thread-like structures were observed penetrating the callus intercellularly and bacteria and bacteroid-like bodies were seen in the cells of the callus tissue. Nitrogenase activity was detected in some samples of calli indicating the nitrogen fixing ability of the infected callus tissue.

## INTRODUCTION

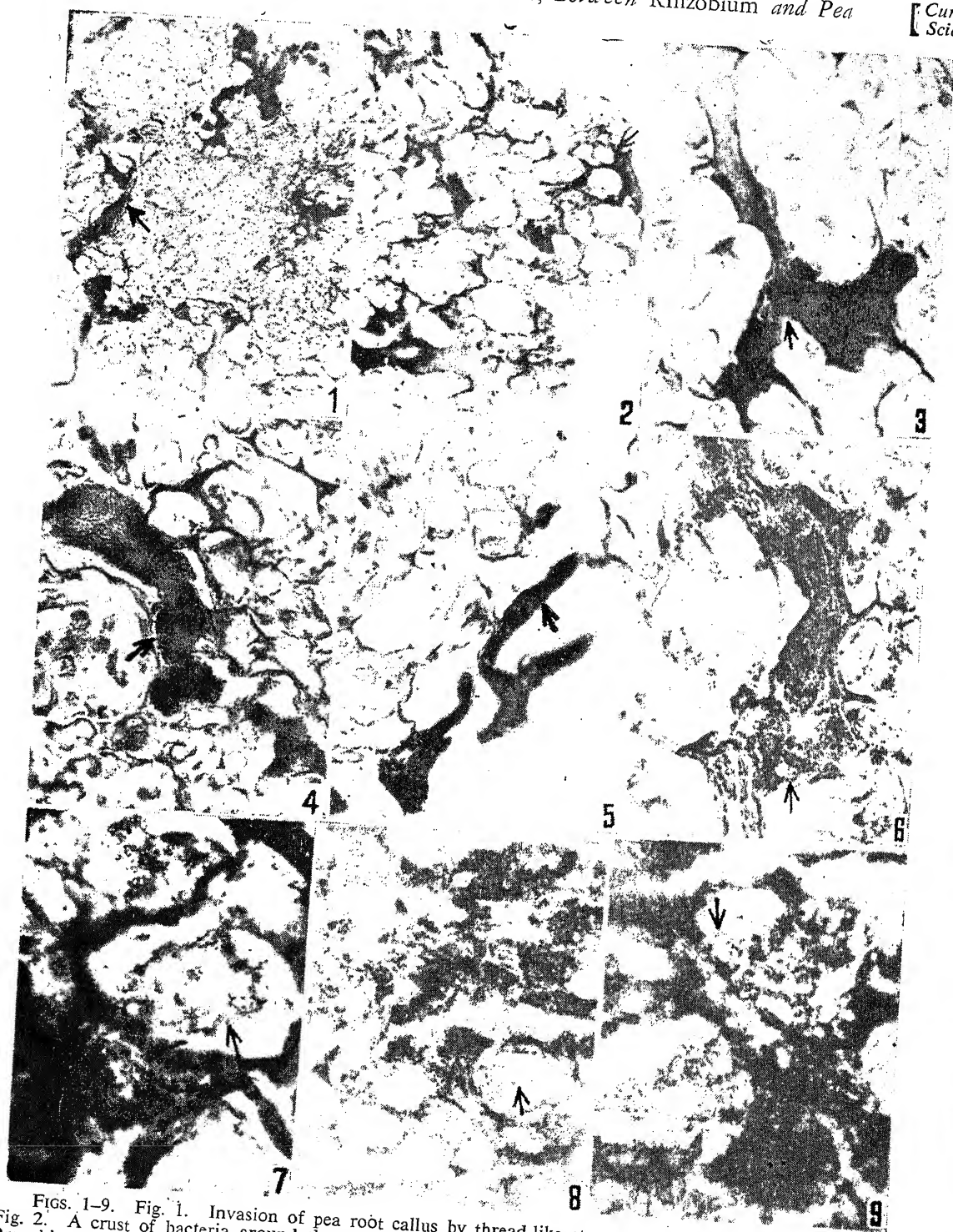
**T**HE formation of infection threads and nodules in legume-*Rhizobium* association has been studied in whole plants<sup>1</sup> and in isolated roots<sup>2</sup> under aseptic conditions. An unsuccessful attempt to establish symbiosis in isolated plant tissue (callus) was made by Veliky and La Rue<sup>3</sup> in 1967. This was followed by a successful attempt by Holsten *et al.*<sup>4</sup> in 1971, using soybean root callus infected with *R. japonicum*. Nitrogenase activity was detected in infected calli (by acetylene reduction technique) and the tissue contained infection thread-like structures and bacteroid-like cells. The results reported in this paper relate to our work with pea root callus infected with *R. leguminosarum*, using the method followed by Holsten *et al.*<sup>4</sup> with slight modifications.

## MATERIALS AND METHODS

Seeds of *Pisum sativum* var. *baunville* were surface sterilised with cetavlon (1%) and germinated on

Murashige-Skoog's<sup>5</sup> (MS) basal medium. The roots were cut off when 2-3 cm long and transferred to MS medium supplemented with 2,4-D and kinetin. Callus formation was observed after 1 month. The callus was transferred to MS basal medium and allowed to remain there for 5 days. Pure cultures of *R. leguminosarum* were isolated from pea root nodules by conventional procedure<sup>6</sup> and maintained on yeast extract mannitol agar (YEMA) slants.

One ml of a YEM broth culture containing actively growing bacteria was transferred to each test-tube containing MS basal liquid medium with actively growing callus and incubated in darkness at  $25 \pm 2^\circ \text{C}$ . Uninoculated calli served as controls. After seven days incubation, the callus mass was washed twice in MS basal liquid medium under aseptic conditions. The solution containing the callus mass was filtered through sterile cheese cloth and calli transferred from the cheese cloth to MS basal solid medium. They were incubated for



FIGS. 1-9. Fig. 1. Invasion of pea root callus by thread-like structures containing bacteria,  $\times 200$ . Fig. 2. A crust of bacteria around the callus from which the threads actually originate,  $\times 400$ . Fig. 3. Branching of threads,  $\times 800$ . Fig. 4. Active intercellular penetration of threads,  $\times 800$ . Fig. 5. Thread-like structures lying loose in the callus,  $\times 800$ . Fig. 6. Tip of a thread showing the release of bacteria into the callus cell,  $\times 800$ . Fig. 7. Liberated bacteria inside the callus cells,  $\times 1,000$ . Fig. 8. Bacteroid-like structures inside the callus cells,  $\times 1,200$ . Fig. 9. Magnified view of callus cells showing bacteroid-like structures,  $\times 1,600$ .

another period of 14 days in dark. Similar treatment was also given to calli which were kept as control.

The calli were fixed in FAA for 24 hours, passed through tertiary butyl alcohol series and embedded in paraffin wax. Microtome sections were cut to 10  $\mu$  and slides were prepared by staining with Safranin-Fast green combination using Canada balsam as the mounting medium. The slides were observed under a light microscope.

Nitrogenase activity of infected calli was analysed by the acetylene reduction technique<sup>4</sup> as follows: The samples were purged with air, stoppered and injected with 0.5 ml of acetylene. The acetylene-ethylene conversion was analysed by gas chromatographic method.

### RESULTS

Microtome sections of infected calli showed infection thread-like structures (hereafter referred to as threads) ramifying the callus tissue (Fig. 1). The threads originated from the bacterial mass which formed a crust around the callus (Fig. 2). Some threads branched repeatedly (Fig. 3). A definite wall could be seen around the thread as in Fig. 4. The figure also indicates intercellular penetration of the thread. Often, threads were observed lying loose near the peripheral cells of the callus (Fig. 5). At certain points, the threads were ruptured at the tip and the bacteria were being liberated as in Fig. 6. Some of the cells of the callus contained bacteria (Fig. 7). At least 5-10% of the peripheral cells of the infected calli were seen filled with bacteroid-like structures (Fig. 8) which were different from those shown in Fig. 7. When magnified, the bacteroid-like structures (Fig. 9) resembled bacteroids in the cells of nodules of intact plants. The data regarding nitrogenase activity as revealed by the reduction of acetylene to ethylene are presented in Table I.

TABLE I  
*Nitrogenase activity in the infected and control samples of pea root calli*

Serial No. of infected calli	Period of incubation	
	1 hour	3 hours
	$\mu$ moles of $C_2H_2 \rightarrow C_2H_4$ per tube	$\mu$ moles of $C_2H_2 \rightarrow C_2H_4$ per tube
1	0.000942	0.001262
2	0.000442	0.000875
3	0.002075	0.002775
4	0.001008	0.001388
5	0.000504	0.000625
Controls (Uninfected)	No activity	No activity

### DISCUSSION

In general, the infection thread-like structures in pea callus were bigger than those observed in *in vivo* in nodulated pea plants and in infected root cell cultures of soybean<sup>4</sup>. The bacteroid-filled cells in pea callus were also usually restricted to the peripheral portion of the callus as observed by Holsten and associates in cell cultures of soybean<sup>4</sup>. In a personal communication to one of us (after seeing our photographs of infected calli), Dr. R. W. F. Hardy of Du Pont Laboratory, U.S.A., comments that our pea callus threads are more abundant and larger than the infection thread-like structures (Pseudo-infection threads) which were observed by Holsten and associates<sup>4</sup> in soybean root callus. He also mentions that the presence of bacteroid-filled cells appears to be a promising feature. Whether or not the thread-like structures observed in these studies could be regarded as a feature arising out of true symbiosis can only be understood by future investigations. However, some of the infected callus cultures were analysed for nitrogenase activity in Dr. Hardy's laboratory. Measurable enzyme activity was detectable. La Rue and Goodchild, in their work on soybean, claim to have successfully established symbiosis using a different methodology. They found continued growth of infected callus which was regarded as a positive sign of symbiosis (La Rue, personal communication). We have also observed continued growth of infected pea calli. Further work on the fine structure and nitrogenase activity of *Rhizobium*-infected calli of different leguminous plants is in progress.

### ACKNOWLEDGEMENT

We are grateful to Dr. R. W. F. Hardy, Research Department, E. I. Du Pont De Nemours Pvt. Ltd., Delaware, U.S.A., for his valuable comments on the observations reported here and for getting the samples of calli analysed for nitrogenase activity in his laboratory. We wish to thank Dr. T. A. La Rue, Prairie Regional Laboratory, Saskatoon, Canada, for making available some of his unpublished work. We would like to express our sincere gratitude to Professor H. Y. Mohan Ram, Head, Department of Botany, University of Delhi, where the tissue culture work was carried out.

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## AN INDIRECT ESTIMATION OF LITTER DISAPPEARANCE IN GRASSLAND STUDY

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## ABSTRACT

An indirect method for estimation of litter disappearance in grassland ecosystem has been evolved and discussed fully in the text.

## INTRODUCTION

THE use of "nylon bags" technique of Shanks and Olson<sup>1</sup> has become a popular method for studying the litter disappearance in grassland and forest ecosystems. By placing litter samples in fine-meshed nylon bags, disappearance can be determined by repeated weighing at intervals. But because of the confinement of the dead leaves, and other plant parts, and restricted entry of the larger soil-floor-fauna, the results do not represent absolute measurements of decomposition of litter under natural conditions. This drawback prompted the present authors to attempt for an alternative reliable estimation.

## MATERIALS AND METHODS

An experiment was conducted near Ratlam (23° 28' N latitude and 74° 58' E longitude) on a two year protected grassfield dominated by *Sehima nervosum* (Rottl.) Stapf, a perennial grass. The topography of the terrain is gently undulating and the climate is monsoonic. Total annual rainfall is 875 mm, most of which occurs during rainy season. Annual mean maximum and minimum temperatures are 31.5° C and 18.0° C respectively. Periodic record of changes in aboveground green biomass, standing dead and litter were taken for one year (June 1971 to May 1972). At each sampling date ten quadrats (size 25 cm × 100 cm) were laid randomly in the experimental site and aboveground plant parts were clipped. The clipping height was ground level. The size of the quadrat was fixed by species area curve method<sup>2-3</sup> and the area obtained was casted in an aforesaid rectangular size to ensure maximum accuracy<sup>4</sup>. The harvested samples were separated species-wise into two categories, viz., aboveground green and standing dead. The ground litter was collected from the harvested plots, brought to the laboratory where it was freed of soil contamination by flotation. All the aforesaid categories were then dried in a hot air oven at 80° C for 24 hours and weighed.

## RESULTS AND DISCUSSION

During rainy season, the growing phase of the vegetation, the *Sehima* grassland community com-

prises of fifteen species including six species of grasses and nine forbs (non-grass). In subsequent winter and summer seasons the species diversity gradually decreases. Aboveground net primary production (AGNPP) comes to about 429.2 g/m<sup>2</sup>/year and is arrived at by summing species-wise positive increments in g/m<sup>2</sup>/year<sup>5</sup>:

$$\text{AGNPP} = \text{Grasses} + \text{Forbs} + \text{Standing dead}^*$$

429.2	344.4	15.2	69.6
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From Table I it is evident that over the year the standing crop of litter decreased from 160 g/m<sup>2</sup> in June to 54 g/m<sup>2</sup> in late August and again in December from 250 g/m<sup>2</sup> to 208 g/m<sup>2</sup> in February. Other periods indicate the increase till it reaches a peak value in May. Obviously the

TABLE I  
Aboveground compartments of the *Sehima*  
community (g/m<sup>2</sup>) 1971-72

Sampling dates	Above-ground live biomass	Standing dead biomass	Litter biomass
June 30 ..	25.19	148.20	160.00
July 15 ..	40.07	120.50	138.00
July 31 ..	59.85	95.70	112.00
Aug. 15 ..	112.12	64.60	78.00
Aug. 31 ..	185.75	86.40	54.00
Sept. 15 ..	306.36	105.40	81.00
Sept. 30 ..	363.14	138.40	107.00
Oct. 31 ..	219.14	229.50	167.00
Nov. 30 ..	158.38	242.30	190.00
Dec. 31 ..	64.12	315.60	250.00
Jan. 31 ..	14.65	302.30	251.00
Feb. 29 ..	5.67	283.30	208.00
March 31 ..	3.66	275.00	214.00
April 30 ..	2.86	240.80	234.00
May 31 ..	1.17	209.10	275.00

period of decline represents the disappearance of the litter and the period in increase represents the input from standing dead and green forbs. Since this input can be estimated from the standing crop of biomass data, the disappearance rate of litter during the study year can be calculated by the following four steps (Table II):

\* Total value only up to peak aboveground live biomass of individual species.

TABLE II

*Transfer of dry matter in different compartments of Sehima community in g/m<sup>2</sup>/year*

1. Contribution by live grasses for increment in standing dead compartment during the year		Positive increment in above-ground live grass biomass (i.e., annual aboveground net production		Contribution to standing dead during active growing season
414.0	=	344.4	+	69.6
2. Input to litter from standing dead compartment		Initial standing dead crop		Increment in standing dead Final standing dead crop
353.1	=	148.2	+	414.0 - 209.1
3. Total increase in litter compartment		Input from standing dead compartment		Contribution from green forbs
368.3	=	353.1	+	15.2
4. Decomposition of litter		Initial standing crop of litter		Increment in litter compartment Final standing crop of litter
253.3	=	160.0	+	368.3 - 275.0

(1) First of all the input of standing dead to the litter compartment will be taken into consideration. Aboveground live grasses contribute to the standing dead compartment during the active growing season, because some live parts die during this season, and after this season all the green aboveground biomass turns gradually into dead which reaches its peak in May. Summation of positive increase due to these two sources represents the total increment in standing dead compartment during the year.

(2) The loss from the standing dead compartment or input to the litter can be obtained by adding this increment in standing dead compartment to its initial standing crop (June) and by subtracting from the final standing crop of dead value (May). The resulting estimate equals the input to litter from standing dead.

(3) The litter compartment has two input sources; the standing dead and the annual input from green forbs as the latter directly moves into litter. The summation of these two values equals the increment in litter compartment over the year.

(4) Finally, the increment in the litter *plus* the initial standing crop of litter (June) *minus* the final standing crop of litter (May) gives the amount of litter disappearing during the year.

Climate plays an important role in litter accumulation. The pounding effect of rain drops during the rainy season and the wind blow effect throughout the year cause the standing dead to decline towards the litter compartment. Thus the dry matter

inventory of the *Sehima* community reveals that annually 253.3 g/m<sup>2</sup> (about 60% of AGNPP) of organic matter disappears through litter decomposition and the minerals get released.

The 'nylon bags' technique, commonly in vogue, involves the measurement of litter disappearance under unnatural conditions such as changed moisture, temperature and aeration. Also the pore size of the nylon bag limits the entry of larger soil-floor-fauna to confined litter kept for decomposition. The present method of indirect estimation for litter decomposition is a modification and simplification of Golley's<sup>6</sup> attempt and seems better in correctness than the 'nylon bags' technique to deal quantitatively with the litter disappearance in natural conditions to assess the dry matter dynamics in grassland ecosystem.

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## LETTERS TO THE EDITOR

FORCE CONSTANTS, CORIOLIS COUPLING  
CONSTANTS AND GENERALISED MEAN  
SQUARE AMPLITUDES OF VIBRATION  
OF  $\text{InCl}_6^{3-}$ 

RECENTLY, Contreras *et al.*<sup>1</sup> have reported the revised vibrational frequencies of the present ion on the basis of octahedral symmetry. In the

The generalised mean square amplitude quantities (Table II) and mean amplitudes of vibration (Table III) were evaluated using Cyvin's method<sup>6</sup> at three temperatures. It is observed that the mean amplitudes of vibrations for bonded as well as non-bonded distances increase with temperature. It is also observed that mean amplitude corresponding to

TABLE I

G.Q.P.F		M.U.B.F.F.		Frequency	Reference
Present	Prev. (Refs. 1, 8)	Present	Prev. (Ref. 8)		
$f_r = 0.903$	.. $f_r = 0.87^*$ (0.949)†	$K = 0.699$	$K = 0.63^*$	$\nu_1 = 275$ (277)	1 9
$f_{rr} = 0.157$	.. $f_{rr} = 0.14^*$ (0.154)†	$k = 0.050$	$k = 0.02^*$	$\nu_2 = 175$ (193)	1 9
$f_{rr'} = 0.050$	.. $f_{rr'} = \dots^*$ (0.010)†	$H = -0.029$	$H = -0.01^*$	$\nu_3 = 245$ (250)	1 9
$f_{ra} - f_{ra''} = 0.051$	.. $f_{ra} = 0.01^*$ (0.089)†	$F = 0.207$	$F = 0.21^*$	$\nu_4 = 150$ (157)	1 9
$f_a - f_{aa''} = 0.111$	.. $f_a = 0.14^*$ (0.089)†	$F' = -0.106$	..	$\nu_5 = 130$ (149)	1 9
$f_{oa} - f_{aa''} = 0.011$	.. $f_{aa} = 0.01^*$ (0.028)†	$h = 0.033$	$h = 0.00^*$	$\nu_6 = 92$ (105)	1 9
$f_{aa'} - f_{aa''} = 0.011$	..	$g = 0.020$	..		

\* Represents force constants due to Ferraro *et al.* (Ref. 8) using the frequency given in Ref. 9.  
† Represents G.V.F.F. reported in reference 1.

present communication, we report the force constants, coriolis coupling constants and mean amplitudes of vibration for  $\text{InCl}_6^{3-}$ , using general quadratic potential function and seven parameter Urey-Bradley force field, which is equivalent to the force field suggested by Venkateshwarlu *et al.*<sup>2</sup>. The equations used by Venkateshwarlu and in modified UBFF are given in the Appendix. In the treatment of Venkateshwarlu<sup>2</sup>, the difficulty arises in solving the secular equations due to the presence of identical force constants  $r^2(H + 0.55F)$  under the three different species ( $F_{1u}$ ,  $F_{2g}$  and  $F_{2u}$ ). This difficulty has been overcome to some extent by introducing two more interaction constants  $K$  and  $h$  in P.E. function. In the present form of P.E. function, an additional interaction term  $F'$  has been included representing the interaction between two angles in the same plane. F-G matrix elements and symmetry co-ordinates<sup>3-4</sup> are the same as those used by earlier workers<sup>5</sup>. From Table I, it is evident that the various force constants are of expected form which may be anticipated in terms of physical parameters.

TABLE II

Generalised mean square amplitudes of vibration  
(in  $\text{\AA}^2 \times 10^{-4}$ ) for  $\text{InCl}_6^{3-}$

Distance	Symbol	T = 0° K	T = 298° K	T = 500° K
In-Cl bonded	$\langle \Delta z^2 \rangle$	27.635	57.230	90.756
	$\langle \Delta x^2 \rangle$	38.342	133.940	220.401
	$\langle \Delta y^2 \rangle$	38.342	133.940	220.401
Cl-Cl linear	$\langle \Delta z^2 \rangle$	47.754	110.651	177.601
	$\langle \Delta x^2 \rangle$	36.576	120.360	197.870
	$\langle \Delta y^2 \rangle$	36.576	120.360	197.870
Cl-Cl non-linear	$\langle \Delta z^2 \rangle$	85.460	237.442	385.311
	$\langle \Delta x^2 \rangle$	70.465	190.042	307.800
	$\langle \Delta y^2 \rangle$	70.030	297.264	492.586

TABLE III

Mean amplitudes of vibration in  $\text{\AA}$  for  $\text{InCl}_6^{3-}$

Distance	T = 0° K	T = 298° K	T = 500° K
In-Cl	0.0526	0.0740	0.0951
Cl-Cl linear	0.0691	0.1052	0.1332
Cl-Cl non-linear	0.0924	0.1540	0.1963

non-linear distance is greater than those for the linear distance. Coriolis coupling constants have been computed employing the method of Cyvin<sup>7</sup>. The coriolis coupling constants for  $(2 \times 2)$   $F_{1u}$  species are  $\xi_1=0.382$ ,  $\xi_2=0.118$ .

Two of us (L. D.) and (D. N. V.) is thankful to C.S.I.R. and U.G.C., New Delhi, respectively for financial assistance.

#### APPENDIX

Force field of Venkateshwarlu

$$F_{11} = K + 4F + k$$

$$F_{22} = K + 0.7F + k$$

$$F_{33} = K + 1.8F - k$$

$$F_{34} = 0.9rF$$

$$F_{44} = r^2(H + 0.55F + 2h)$$

$$F_{55} = r^2(H + 0.55F - 2g)$$

$$F_{66} = r^2(H + 0.55F - 2g)$$

M.U.B.F.F.

$$F_{11} = K + 4F + k$$

$$F_{22} = K + F + 3F' + k$$

$$F_{33} = K + 2F + 2F' - k$$

$$F_{34} = F + F'$$

$$F_{44} = H + F/2 - 3/2F' + 2h$$

$$F_{55} = H + F/2 - F'/2 - 2g$$

$$F_{66} = H + F/2 - F'/2 - 2h$$

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#### THE CRYSTAL STRUCTURE OF CALCIUM PHTHALATE MONOHYDRATE $\text{Ca}_2\text{C}_8\text{H}_4\text{O}_4 \cdot \text{H}_2\text{O}$

THE crystal structure of calcium phthalate monohydrate,  $\text{Ca}_2\text{C}_8\text{H}_4\text{O}_4 \cdot \text{H}_2\text{O}$ , has been determined from single crystal X-ray diffraction data.

Unit Cell: Monoclinic,  $a = 11.28$ ,  $b = 6.67$ ,  $c = 11.91$  Å,  $\beta = 99.3^\circ$ ,  $Z = 4$ ,  $\rho_{\text{calc}} = 1.68$  g/ml,  $\rho_{\text{mea}} = 1.67$  g/ml; space group  $P2_1$ . This is in agreement with values reported earlier<sup>1</sup>.

**Structure Determination.**—The structure was determined by the heavy atom technique using three-dimensional Patterson synthesis, followed by iterative Fourier refinements in projections  $\langle [001]$ ,  $[010]$ , and  $[100] \rangle$ .

**Crystal Structure.**—In the crystal COOH groups of phthalate ions are linked via the water molecule along  $[001]$  with hydrogen bonds of 3.12 and 2.96 Å, the angle between these two bonds being  $78.5^\circ$  (Fig. 1). There are six oxygen atoms around the

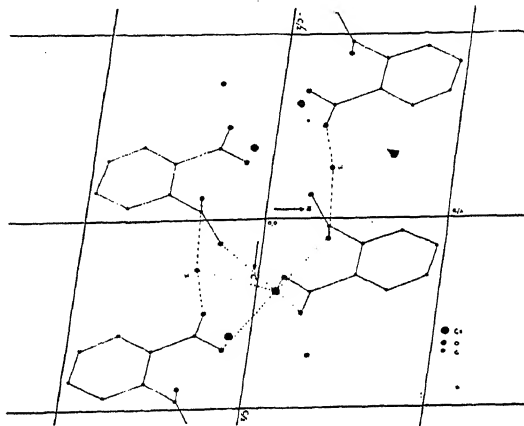


FIG. 1. The crystal structure looking down  $[010]$ .

Ca ion with Ca—O distances ranging from 2.31 to 2.60 Å with an average value of 2.47 Å. The stereochemistry of the molecule is normal, though a little different from that reported by other workers<sup>3,2</sup>. The refinement of the structure is under progress.

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#### THE CRYSTAL STRUCTURE OF *p*-BROMO-ACETOPHENONE, $\text{Br.C}_6\text{H}_4\text{COCH}_3$

THE crystal structure of *p*-bromoacetophenone has been determined by X-ray diffraction as a check to confirm the stereochemistry of the acetophenone molecule<sup>1</sup>.

Crystal data: Monoclinic with

$a = 19.16$ ,  $b = 7.10$ ,  $c = 6.02$  Å,  
 $\beta = 102.5^\circ$ ,  $Z = 4$ ,  $\rho_{\text{calc}} = 1.65$  gm/ml,  
 $\rho_{\text{obs}} = 1.64$  gm/ml,  $\mu = 70.4$  cm<sup>-1</sup>  
for  $\text{CuK}\alpha$  radiation.



Space group: The following systematic absences were observed.

$$hkl: h + k \neq 2n;$$

$$hol: l \neq 2n \ (h \neq 2n);$$

$$oko: k \neq 2n.$$

These conditions lead to either *Cc* or *C2/c*. As the molecule possesses no centre or plane of symmetry and as there are only four molecules in the unit cell, the possibility of molecules occupying special positions in the space group *C2/c* can be ruled out. The correct space group is therefore *Cc*.

*Crystal structure.*—The structure was solved by the heavy atom technique and refined by Fourier methods. The view of the structure down [100] is given in Fig. 1. In the crystal the molecules

The structure is, therefore, loosely bound and accounts for the volatility of the compound. The aceto-group is rotated by  $3.5^\circ$  from the plane of the aromatic ring. This is close to the value of  $6^\circ$  found in *p*-oxi-acetophenone<sup>2</sup>.

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### EFFECT OF PLURONIC F-68 ON SURFACE TENSION AND VISCOSITY OF BLOOD AND PLASMA

THE rheological properties of blood depend on the surface characteristics of red blood cells and plasma<sup>1</sup>. The surface properties can be altered by the use of surfactants. Pluronic F-68 (polyoxypropylene polyoxyethylene glycol) is a non toxic, non-ionic surface active agent with a molecular weight<sup>2</sup> 8350. This surfactant had been used to enhance circulation. Recently it was suggested as a therapeutic agent in hemorrhagic shock and it was observed that the addition of 0.4 gm% pluronic F-68 reduces the surface tension of whole blood and is effective in restoring microcirculation<sup>3</sup>. The reduction in viscosity of blood due to F-68 was also reported by Frederick, L. *et al.*<sup>4</sup>. However there is no experimental evidence to show which component of the blood is effective for the decrease in surface tension and viscosity of whole blood. Hence the present work was undertaken with a view to evaluate the potentialities of two important components, *viz.*, red blood cells and plasma in reducing the viscosity and surface tension of whole blood.

Blood samples were obtained from healthy donors of Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, by routine blood bank procedures, anti-coagulated in citrate solution. The blood samples used for experiments were of some haematocrit and protein content. The surface tension of whole blood was determined using DuNouy tensiometer<sup>5</sup> at  $30 \pm 1^\circ \text{C}$ . The relative viscosity of the same blood with respect to water was determined at  $30 \pm 1^\circ \text{C}$  using microviscometer<sup>6</sup>. 0.4 gm% pluronic F-68 added to the above samples and its surface tension and viscosity were determined as before. The plasma was separated from normal blood by centrifuging and its surface tension and relative viscosity were determined before and after adding pluronic F-68.

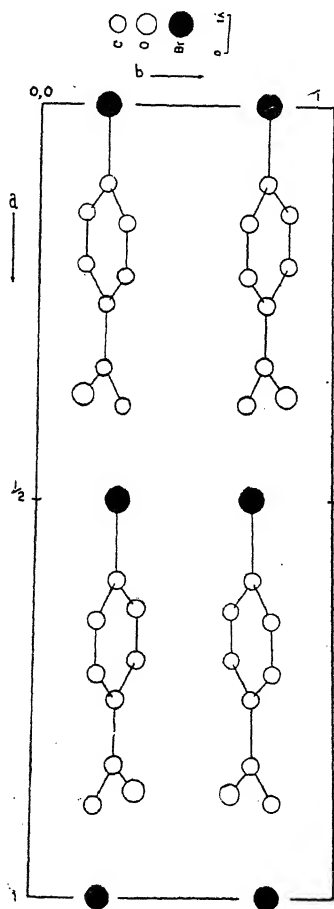


FIG. 1.

are arranged in two sets of layers being related among themselves by the 'c' glide operation. These two sets make an angle of nearly  $60^\circ$  with each other. The intermolecular contacts are the usual van der Waals ones, ranging from 3.52 to 4.3 Å.

Six samples were taken in each case and the experiment was repeated five times. The observations are given in Table I.

TABLE I  
Surface tension and viscosity of normal and pluronic added samples

Measurement		Whole Blood	Plasma
Surface tension (dynes/cm)	normal	53.36	53.10
		$\pm 1.64$	$\pm 1.02$
	normal	43.40	44.50
	+F-68	$\pm 0.70$	$\pm 0.50$
Relative viscosity (w.r. to water)	P value	$< .001$	$< .001$
	normal	5.31	1.68
		$\pm 0.30$	$\pm 0.04$
	normal	4.61	1.70
	+F-68	$\pm 0.01$	$\pm 0.04$
	P value	$< .001$	$> 0.05$

Values are mean  $\pm$  S.D. Number of samples = 6

There is no significant difference in the surface tension of normal blood and plasma. After adding the surfactant the reduction in surface tension is the same in both cases. From these results it can be inferred that the reduction of surface tension of whole blood is probably due to reduction of surface tension of plasma brought about by the surfactant. This reduction of surface tension may be due to the weakening of the cohesive forces brought about by addition of the surfactant.

The relative viscosity of whole blood was significantly reduced ( $p < .001$ ) Table I) in the presence of pluronic F-68 but the relative viscosity of plasma was not altered. It is not unreasonable to infer that the pluronic F-68 is not effective in changing the viscosity of plasma at this concentration.

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## OCCURRENCE OF A REMNANT PRIMORDIAL SIALIC CRUST IN NORTHEASTERN MINNESOTA, U.S.A.

**Introduction.**—The purpose of this note is three-fold: (1) to report the author's discovery in the Minnesota segment of the Canadian Shield of a rock unit that is unlike any known petrologic unit in the area, (2) to suggest on geologic considerations that it is a sialic basement on which one of Earth's oldest mafic volcanic piles was deposited, and hence it is a remnant of the Earth's primordial sialic crust, and (3) to propose that the area holds the key to unlock the mysteries of proto-crustal evolution in very Early Precambrian times, some 3500-4000 million years ago.

**Location.**—The rock unit consisting of low-lying outcrops occurs in a highly swampy country around Buck Lake in Townships 58 N and 59 N, and Ranges 22 W and 23 W of Itasca County in northeastern Minnesota (U.S.G.S. 7.5 minute O'Leary Lake quadrangle). It was discovered by the author during geologic mapping of the area during the summers of 1968 and 1969. The area is accessible from Nashauk, about 12 miles to the south, via County Road 65.

**Petrography.**—The preponderant rock types in the unit are hornblende tonalitic gneiss, biotite tonalitic gneiss, hornblende mesotonalite, and biotite leucotonalite. These are associated with small bodies of amphibolite, hornblendite, agmatite, granitoid, aplite, and pegmatoid.

**Petrogenesis.**—Field observations, and thin section studies reveal (1) a strong deformational imprint on all the constituent rock types of the unit, and (2) an origin of the complex through metamorphism of a granitic plutonite, and of minor mafic bodies injected into it, under amphibolite-facies conditions, accompanied by metasomatism, migmatization, and metamorphic differentiation.

**Stratigraphy.**—The rock unit, herein designated the 'Buck Lake Tonalitic Gneiss Complex' ('BLTGC', in abbreviated form), apparently underlies a large northwest-trending body of pillowed mafic metavolcanic rocks. Judged from pillow tops near the base of the metavolcanic unit, which consistently face northeastward, it appears that

the metavolcanic unit stratigraphically overlies the 'BLTGC'.

**Correlation and age.**—The 'BLTGC' is unlike any known petrologic and chronostratigraphic unit in the area for the following reasons: The oldest granitic rocks of the region, namely, the various phases of the 2700 million year old Giants Range batholith<sup>1</sup> are practically undeformed rocks, and post-date the mafic metavolcanic succession that overlies the 'BLTGC'. A correlation of the 'BLTGC' with the two tonalitic components of the Giants Range batholith<sup>1</sup> is also not possible because detailed geochemical studies of rubidium, strontium, barium, lead, zinc, and niobium distributions in the Giants Range tonalitic rocks show that they formed by anatexis of volcanogenic metasedimentary rocks. A consideration of these aspects when viewed against the lower metamorphic grade of the post-'BLTGC' mafic metavolcanic rocks (greenschist facies), relative to the higher metamorphic grade of the 'BLTGC' (amphibolite facies), suggests that the 'BLTGC' represents a metamorphosed granitic intrusive sequence that pre-dates the intrusive 2700 million year old granitic rocks of the Giants Range batholith.

**The 'BLTGC' as a remnant primordial sialic crust.**—The salient features of the petrology, correlation, age, and the stratigraphic position of the 'BLTGC' below one of the oldest mafic volcanic piles indicate that it is possibly a remnant primordial sialic crust of the Earth. Such an interpretation is consistent with recent considerations of the Earth's early thermal history which suggest that the earliest crustal fragments would be granitic<sup>2</sup>. A powerful argument against a primordial sialic crust for the Earth, however, is stated to be the lack of any evidence of lowermost Early Precambrian mafic volcanic assemblages resting on granitic rocks<sup>3</sup>. Therefore, the author considers his discovery of the 'Buck Lake Tonalitic Gneiss Complex' to hold the key to unlock the mysteries of protocrustal evolution in very Early Precambrian times, some 3500–4000 million years ago. A detailed account of this complex, together with a critical comparison of it with the 3200–3400 million year old 'Ancient Tonalitic Gneisses' of Swaziland, a discussion of the possibility of its being an analogue of the oldest dated rocks of the Earth's crust, namely, the recently discovered 3800 million year old Amitsoq gneisses of the Godthab District of West Greenland, and the significance of the 'BLTGC' to models of early crustal evolution, will be published elsewhere.

**Acknowledgement.**—Dr. Paul Sims suggested geological and petrological studies in the western part of the Giants Range batholith,

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## MIDDLE CARBONIFEROUS FOSSIL BED FROM WESTERN RAJASTHAN

DURING the course of recent field work in Bap area (27° 25' : 72° 25') about 150 km north-west of Jodhpur, the authors found the exposures of a new fossil horizon, underlying the Bap boulder bed. This is the first known fossiliferous horizon in Peninsular India which directly underlies Upper Carboniferous tillite. It is distinctly older than the Talchir Glaciation, and consequently older than any fossiliferous bed reported from Peninsular India, so far<sup>1-12</sup>.

The exposure is seen in a depression about 100 metres west of Bap-Badhaura, road and about a kilometre north of Bari Dhani (3 km south-west of Bap village).

The fossils occur in two bands each of about 20–30 cm in thickness and a more than 3 m thick violet and light brown siliceous limestone dipping at an angle of 10°–15° in S. 25° W direction.

The contact between the tillite and the limestone is not parallel to the bedding plane of the limestone, so it is concluded that the tillite overlies the limestone unconformably. The general stratigraphical sequence of the area is as follows:

- Badhaura Formation.....L. Permian.
- .....Disconformity.....
- Bap Boulder Bed.....U. Carboniferous.
- .....Unconformity.....
- Unfossiliferous band of light brown limestone—50 cm
- Upper fossiliferous band of light brown limestone—20 cm
- Unfossiliferous band of violet and light brown limestone—10 m
- Lower fossiliferous band of violet brown and light brown—30 cm

limestone.

The lower fossiliferous band is violet and light brown in colour and thickly packed with *Nucula beyrichi* and *Nucula girtyi*.

The upper fossiliferous band is light brown coloured limestone containing lamellibranchs, gastro-

podis and cephalopods with an abundance of *Nucula* :

Lamellibranchs

*Nucula beyrichi*

*Nucula girtyi*

Gastropods

*Auripygma virgatus*

*Strapdrollus strapdrollus*

Cephalopod

*Liroceras liratum*

From these two bands an exhaustive collection of fossils has been made and a detailed study and identification of the entire collection is in progress.

*Age of the Bed*

The lower fossiliferous band has at least two species of *Nucula*, i.e.,

(i) *N. beyrichi*.

(ii) *N. girtyi*.

The former is confined to the Pennsylvanian period and the latter has a range from Silurian to Recent. The fossils, therefore, suggest an age of Upper Carboniferous for the band.

The upper fossiliferous band has, along with the above-mentioned two *Nucula* species :

(i) *Auripygma virgatus*

(ii) *Liroceras liratum*

(iii) *Strapdrollus strapdrollus*

The first is restricted to the Mississippian period (Lower Carboniferous) whereas the second is limited to the Pennsylvanian period (Upper Carboniferous) and *Strapdrollus strapdrollus* extends from Lower to Upper Carboniferous.

This bed has a mixed assemblage of Mississippian as well as Pennsylvanian North American Index Fossils. The presence of *Auripygma virgatus* in upper limestone band with Pennsylvanian fossils in the lower band suggests that in India these fossils cannot be used to separate Mississippian beds from the Pennsylvanian. The fossil assemblage in these beds indicate Carboniferous age.

This bed is underlying Bap boulder bed unconfirmably which is assigned Upper Carboniferous age" so these fossil beds may be middle carboniferous.

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#### QUARTZINE AND LUTECITE AS ENVIRONMENTAL INDICATORS IN SIRBAN LIMESTONE OF RAISI, J AND K STATE

SIRBAN Limestone inlier in the Murrie belt of J and K State near the town of Raisi (33° 41' : 74° 50') presents a stratigraphic thickness of about 4,500 feet. A detailed mineralogical, petrographic and sedimentological study of this limestone revealed that the rocks are essentially dolomites with a flysch-like succession of thin limestone and shale at the top (Rao and Khan, 1971). The rocks show extensive silica diagenesis. A time-trend analysis of dolomite proportion in a vertical profile near Raisi (Rao, 1973) showed that silica replacement of dolomites is somewhat cyclic. Silica in these rocks at the outcrop level occurs in two forms, viz., thin replacement bands of a few inches across, especially in the lower portion of the section, and as replacement masses and pore-filling cement in the upper part of the section. A microscopic examination of these chert occurrences revealed three kinds of quartz : (1) microcrystalline quartz, (2) chalcedonic/spherulitic quartz and (3) mosaic quartz (Rao and Khan *op. cit.*). Various combinations of these three kinds of quartz both in the chert bands and pore-filling cements from periphery to the centre may be observed, namely, (1) microcrystalline quartz-chalcedonic quartz-mosaic quartz, (2) microcrystalline quartz-mosaic quartz, (3) chalcedonic quartz-mosaic quartz, (4) chalcedonic quartz-microcrystalline quartz and *vice versa*, or only chalcedonic microcrystalline or mosaic quartz. Figure 1 shows chalcedonic quartz going into mosaic quartz in an intergranular cavity

of a pellet rock, and Fig. 2 presents microcrystalline quartz in a chert band going into chalcedonic quartz wherein the quartz units are elongated and broad, like dogtooth spar.



FIG. 1. Microphotograph of a quartz pool occurring as a pore-filling cement in a pelletoid rock of Sirban Limestone of Raisi, J and K State. Note lutecite along the periphery of the pool gradually giving place to megaquartz at the centre. Crossed nicols,  $\times 45$ .

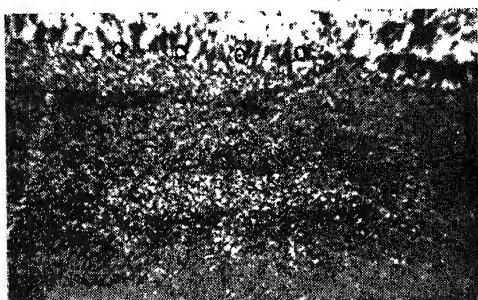


FIG. 2. Microphotograph of a chert band giving place to quartzine towards the top. Note quartzine units are elongated and somewhat stout. Cross nicols,  $\times 45$ .

Optically there are two kinds of chalcedony, length fast, that is the fast ray coinciding with the 'C' crystallographic axis of the grain, and length slow where the slow ray coincides with the 'C' crystallographic axis of the grain; here the mineral is called quartzine. Lutecite is a chalcedonic quartz where the slow ray makes an angle of  $30^\circ$  with the crystallographic "C" axis of the grain. Folk and Pittman (1971) give an extensive discussion of the occurrence of quartzine and lutecite in carbonate rocks and make out a point that they invariably represent the replacement of original alkaline and sulphate minerals and thus suggest, evaporite, sabkha or playa lake environment even though where any direct evidence as to the occurrence of evaporitic minerals is not available. Quartzine and lutecite represent replacement of original evaporitic minerals. With this point in view, the author made a thorough search for quartzine and lutecite in the chert

occurrences of the Sirban Limestone of Raisi. To his surprise, he found many bands and pools of chert showing the occurrence of these two minerals. Figure 1 exhibits a pool of silica occurring as a pore-filling cement. Here lutecite gradually gives place to centrally disposed megaquartz. Figure 2 is of a chert band where microcrystalline quartz is gradually giving place to quartzine. The crystals are elongated and stout, so characteristic of quartzine occurrences (Folk and Pittman *op. cit.*). Rao and Khan (*op. cit.*) documented many evidences in favour of sabkha origin of the dolomites of Raisi. May be the occurrence of lutecite and quartzine above reported represent the replacement of original sulphate minerals, even though no direct evidence as to their presence in the Sirban Limestone of Raisi is now registered. Then one may regard that the occurrence of quartzine and lutecite in Sirban Limestone of Raisi adds further evidence in support of the earlier conclusions of Rao and Khan (1970) that these rocks are of sabkha origin.

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#### SIGNIFICANCE OF THE DEVELOPING VERTEBRAL COLUMN OF *PELODYTES* *PUNCTATUS* TADPOLE

OF all vertebrates, the Anura have the shortest vertebral column; it consists of not more than nine free vertebrae and a rigid bony rod, the urostyle or os-coccygeum, representing the post-sacral vertebrae. That the urostyle is formed of fused post-sacral vertebrae is indicated in the adult by nerve aperture and sometimes, as neural arches anteriorly.

*Pelodytes* tadpoles were collected in Georgia (USSR) and fixed in Bouin's fluid and preserved in 70% alcohol. This material was brought to India by Prof. L. S. Ramaswami with a view to compare the development of the vertebral column in *Pelodytes* with that of Indian species. He placed the material unreservedly at my disposal. Sections were cut  $6\mu$  after routine paraffin embedding. On

comparing *Pelodytes* tadpoles with Indian ones (*Rana* and *Bufo*), the vertebral column showed some interesting features. *Pelodytes* showed twelve neural arches (Fig. 1) as compared to ten of *Rana* or *Bufo*.

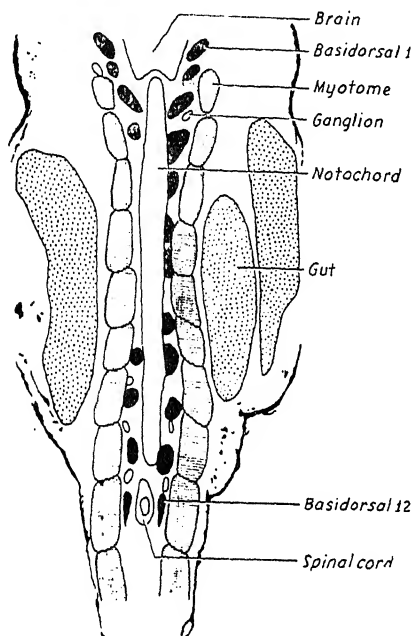


FIG. 1. Diagrammatic horizontal section of *Pelodytes* tadpole (20 mm),  $\times 35$ .

The Pelobatinae (Europe and North America) is diagnosed by their anchylosed sacro-coccyx except in *Pelodytes* which has a single condyle<sup>1</sup>. The pattern of vertebral column of this family has never been reported unequivocally. In this not only is the pattern of the presacral elements unpredictable<sup>2-5</sup> but the nature of the coccygeal articulation, to which both Nicholls and Noble accorded much significance, may be monocondylar<sup>6-8</sup> or bicondylar<sup>9-12</sup> in different individuals of the same species (*Pelodytes punctatus*). Similarly, the number of vertebrae and the size of urostyle have been subjects of wide controversy. A fossil tadpole shows the caudal fin fold preserved as a fairly distinct imprint, and strangely enough, a series of about twenty free vertebral elements extending through body and tail upto its tips<sup>13</sup>. This fossil tadpole seems to be the earliest one known so far. The term 'urostyle' is scarcely applicable since the composing bony units never were caudal. In *Rana* and *Bufo* the ninth is the only sacral vertebra, connecting the ilea by means of transverse

diapophyses. *Pelobates*, *Pipa* and *Hymenochirus* have normally two sacra. *Bombinator* is still in a transitional condition, normally the ninth is the only sacral, but in certain individuals the tenth has also diapophyses weak and long enough to reach the ilea. The eleventh is free in larva, but it fuses later with the twelfth, which already belongs to the coccyx. The information available is sparse and conflicting; some authorities<sup>14-18</sup>, interpret the urostyle as formed of anchylosed vertebrae; others<sup>19,20</sup> deny that it is segmental at all, whilst a third school<sup>21-23</sup> concludes that it represents the fusion of vertebral units with an undivided sub-notochordal rod of cartilage, the hypochord. This disagreement stems from the fact that the majority of Salientia do not normally develop caudal vertebrae so that interpretation of urostyle elements which develop proximally in an otherwise regressing tail is made extremely difficult. This species like *Pelobates*, *Pipa* and *Hymenochirus* has two sacra and the eleventh is free in the larva which later on fuses with twelfth to form coccyx.

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### FOETAL MEMBRANES IN THE INDIAN HORSE-SHOE BAT, *RHINOLOPHUS ROUXI* (TEMMINCK)

A PERUSAL of the literature on the embryology of bats reveals that there is no information about the development and the structure of the foetal membranes of any member of the family Rhinolophidae except for a casual reference to the yolk sac of *Rhinolophus hipposideros* by Van der Sprenkel (1932) and to the presence of a discoidal endotheliochorial placenta in "rhinolophids" by Hamlett (1934). The present report embodies the description of the arrangement and the structure of the foetal membranes of *Rhinolophus rouxi* at full term.

The uterus is bicornuate, but a single embryo is carried invariably in the right cornu during each pregnancy. At full term the pregnant uterine cornu measures 3 cm in the tubo-cervical axis and 2.3 cm in cross-section. The foetus lies in the uterus in such a manner that its cranio-caudal axis is parallel to the tubo-cervical axis of the uterus, the head of the foetus lies towards the cervix, and the dorsal side of the foetus is towards the median side of the uterus. Due to this orientation of the foetus the umbilical cord bends towards the placental disc which is mesometrially located.

The general arrangement of the foetal membranes at term is indicated in Fig. 1. The amnion is a thin bilaminar membrane which is closely adherent to the body of the foetus on all the sides except near the cranial and the caudal flexures where a small part of the fluid filled amniotic cavity persists.

The yolk sac lies adjacent to the placental disc on the mesometrial side of the uterus in the form of a collapsed bag with intensely folded walls. Consequently the cavity of the yolk sac is reduced to streak-like spaces. The endodermal cells have hypertrophied and are cubical to columnar each with a centrally placed vesicular nucleus with a darkly staining nucleolus (Fig. 2). The endodermal cells form a continuous lining for the remnants of the yolk-sac cavity. The mesodermal cells are fusiform or polygonal and contain darkly staining nuclei,

The umbilical cord is 1 cm long and has undergone coiling two or three times. Five blood vessels—two umbilical arteries, an umbilical vein, a vitelline artery and a vitelline vein—and a narrow endodermal allantoic duct are seen in transverse sections of the umbilical cord (Fig. 3).

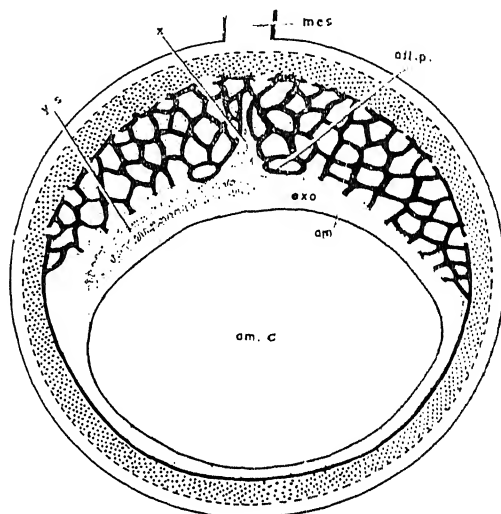


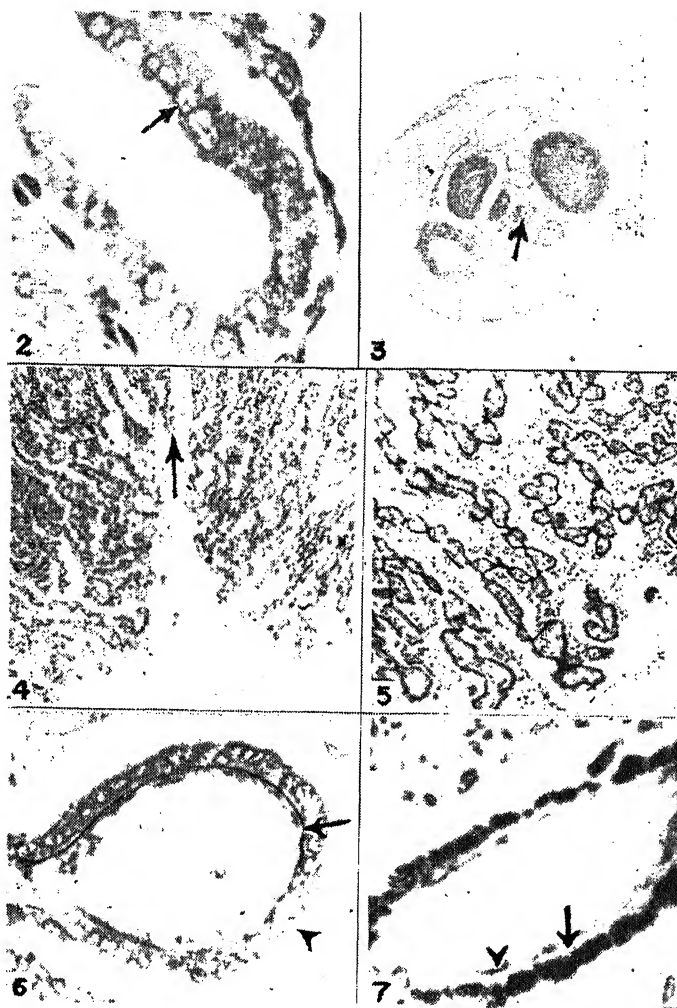
FIG. 1. Semischematic diagram to illustrate the general arrangement of the foetal membranes at term of *Rhinolophus rouxi*. am : amnion ; am. c : amniotic cavity ; ch-all. pl : chorio-allantoic placenta ; exo : exocoelom ; mes : mesometrium ; x : cleft in the placenta ; y.s : yolk sac.

The chorio-allantoic placenta is in the form of a concavo-convex disc located on the mesometrial side of the uterus. It is 1.5 cm in diameter and 6 mm thick in its centre. There is a deep and wide cleft in the centre of the placental disc. Hence, in transverse sections the placenta appears to be made up of 2 discs (Figs. 1 and 4). The umbilical cord is inserted to the centre of the cleft in the placenta.

Histologically the chorio-allantoic placenta consists of a complex three-dimensional network of highly convoluted tubules, the meshes of the network being occupied by allantoic mesenchyme and foetal capillaries (Fig. 5). Each placental tubule typically consists of a central maternal blood capillary with a distinct lining of endothelial cells, a PAS-positive interstitial membrane and a well-defined cytotrophoblastic layer with regularly arranged cubical cells each with a large vesicular nucleus (Fig. 6). In the larger placental tubules near the foetal border of the placenta there is an enucleate cytoplasmic lamina between the endothelial lining and the cytotrophoblastic layer (Fig. 7). This is the

remnant of the syncytiotrophoblast. However, this is absent from the finer tubules in the deeper regions

vasomonochorial in those regions where only the cytotrophoblast is present.



Figs. 2 to 7. Fig 2. A part of the yolk-sac splanchnopleure to show the hypertrophied endodermal cells (arrow),  $\times 325$ . Fig. 3. Transverse section of the umbilical cord at term. The arrow points toward the allantoic duct,  $\times 22$ . Fig. 4. Part of the placental disc to show the cleft (arrow) in the centre of the disc.,  $\times 20$ . Fig. 5. Part of the allantoic placenta to show the network of convoluted placental tubules embedded in allantoic mesenchyme,  $\times 40$ . Fig. 6. Part of the placental tubule (PAS-staining) showing the cytotrophoblast (arrow head) and the PAS-positive interstitial membrane (arrow). Note also the endothelial cells lying on the maternal side of the interstitial membrane,  $\times 325$ . Fig. 7. Part of the placental tubule to show the thin enucleate lamina of the syncytiotrophoblast (arrow) between the endothelial cells (arrow head) and the darkly staining layer of cytotrophoblast,  $\times 180$ .

of the placenta where only the cytotrophoblast forms the foetal component of the placental tubules. Hence, during the final stages of gestation the composition of the placental barrier appears to be different at different regions, being vasodichorial in the larger placental tubules where both cytotrophoblast and the syncytiotrophoblast are present, and

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# PRESERVATION OF BUFFALO SEMEN AT ROOM TEMPERATURE (20°-28° C) IN RUSSIAN DILUTOR

THE presentation of buffalo semen either at room or at refrigerator temperature is still a problem.

20°-28° C temperature. The semen was examined for progressive and per cent motile spermatozoa at 0 hr and subsequently at every 24 hourly interval upto 168 hours using separate vials each time. The results have been summarised below :

TABLE I

Showing average motility grade and per cent motile spermatozoa

Motility of spermatozoa	Fresh semen	Hours of storage							
		0	24	48	72	96	120	144	168
Progressive*	4.00	4.00	3.63	3.38	3.00	2.88	2.00	1.25	0.75
Per cent	76.25	76.25	75.00	71.25	66.25	63.75	43.75	26.25	20.00

\* Quality of motility based on a scale of 0 for no motility to 5 for excellent progressive motility (Norman *et al.*, 1958<sup>2</sup>).

The semen of buffalo bulls extended in diluents commonly used, *i.e.*, glucose bicarbonate and egg yolk citrate, etc., can be used for artificial insemination (A.I.) only upto 2-3 days of storage (Singh and Saxena<sup>1</sup>). The problem is further aggravated by non-availability of ice and carriage of thermos in rural areas.

Earlier investigations conducted by Norman *et al.*<sup>2</sup> revealed that buffalo semen could be extended in Coconut Milk Extender (CME) and stored at 20°-33° C. Such semen was found suitable for A.I. upto about seven days of storage. However, implementation of CME posed the problem of import of essential antibiotics, antifungal agents and enzymes. Later, studies of Saxena *et al.*<sup>3</sup> revealed that semen of Jersey bulls was fit for A.I. upto 3-5 days of its storage at 20°-28° C in CME and Russian Dilutor (RD).

The composition of RD (Milovanov *et al.*, 1964)<sup>4</sup> is as follows :

I. Pot. dihydrogen phosphate	..	720 mg.
Glass distilled water	..	100 ml.
II. Sodium citrate dihydrate	..	20.276 g.
Glucose	..	5.700 g.
Sodium bicarbonate	..	1.260 g.
Penicillin G. Sodium	..	600 mg.
Dihydro streptomycin sulphate	..	1.0 g.
Sulphanilamide	..	3.0 g.
Glass distilled water	..	900 ml.

One part of I and 9 parts of II are mixed and 11% egg yolk is added before dilution.

The buffalo semen was extended in RD at the dilution rate of 1 : 30. This was then filled in 2 ml capacity, sterilised plastic vials with caps tightly fitted over them. Care was taken to avoid air space. The vials were kept in a dark place at

The results revealed that the buffalo semen extended in RD and stored at 20°-28° C room temperature maintained satisfactory motility for A.I. upto 72-96 hours of storage.

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## VARIETAL DIFFERENCES OF RICE TO SALT TOLERANCE AT GERMINATION

In crop production in areas where salinity is a major factor, the ability of a given species or variety to germinate and have a good stand is frequently a limiting factor. It has been reported that salinity delays germination. Decrease in germination with increase in concentration of salt in the medium has been reported in rice (Narale *et al.*,

1969). Varietal differences in salt tolerance were observed at germination and seedling growth in rice by Bhattacharyya (1965).

The present study was undertaken to analyse the relative salt tolerance of seven varieties of rice at germination stage to find out the relative varietal differences of rice to salt tolerance.

The following seven varieties of rice (*O. sativa*) were selected for the study :

- |              |               |
|--------------|---------------|
| 1. Getu,     | 4. Kalajira,  |
| 2. Sadamota, | 5. Hamsa,     |
| 3. Rupsal    | 6. Badsabhog, |
|              | 7. Basmati.   |

The experiment was carried out in the laboratory at room temperature and solution culture technique was followed. Sodium chloride solutions of 1.0 and 1.5% concentrations were used. Deionized water served as control.

For germination studies, 100 rice seeds for each variety (four replications) were kept in petridishes with filter-paper (Whatman No. 1) in which the solution of sodium chloride was added. The germination count was taken after two days and continued upto 9th day at 24 hour interval. The filter-papers and the salt solutions were changed every 24 hours to check the effect of changing salt concentration owing to evaporation. The seeds were designated as 'germinated' only when they exhibited about 0.5 cm of coleoptile growth. Along with these a control was run for each variety in the same manner except that deionized water was used instead of salt solution.

Per cent reduction in germination was calculated by taking into account the number of seeds that germinated under non-saline condition for each variety. The data (presented in Fig. 1) were analysed statistically. Analysis of variance is presented in Table I.

TABLE I  
Analysis of variance of germination percentage

Source	Degree of freedom	Variance	Treatment variance E. Error variance
Treatment ..	2	37684	197.2†
Varieties ..	6	2974	15.5†
Replicates ..	3	51	0.2
Var. × Treat. ..	12	690	3.6*
Error ..	60	191	..
Total ..	83	..	..

#### RESULT

From Fig. 1 it becomes clear that salt solution of 1% did not reduce the germination per cent beyond 10 in Getu, Sadamota, Rupsal and Kalajira whereas in Hamsa, Badsabhog and Basmati the

per cent reduction in germination was greatly increased by salt concentration of 1%. Salt concentration of 1.5% considerably reduced the germination percentage. A 50% reduction was noted in Getu and Sadamota. 100% reduction in germination was observed in Badsabhog and Basmati.

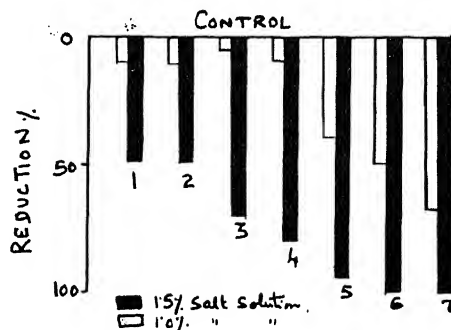


FIG. 1. Effect of salt solution on germination. (1) Getu, (2) Sadamota, (3) Rupsal, (4) Kalajira, (5) Hamsa, (6) Badsabhog, (7) Basmati.

Statistical analysis of the data reveals the factor, variety and treatment as well as their interaction to be highly significant (Table I).

The present study clearly shows that the rice varieties studied exhibit a relatively variable salt susceptibility at the germination stage. Such varietal differences were also reported in cereals by Maliwal and Paliwal (1967), Sarin and Narayan (1968) and Bhumbra *et al.* (1968). The salt supply increases the per cent reduction in germination by delaying it. It is evident that Basmati is highly susceptible to salt concentration of even 1% while in Getu and Sadamota reduction in germination per cent does not exceed 50 even at a salt concentration of 1.5%. Rupsal and Kalajira show considerable amount of salt tolerance at 1% salt solution.

Getu and Sadamota exhibit a greater degree of salt tolerance during germination stage in comparison to the other varieties.

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## INFLUENCE OF LIGHT AND TEMPERATURE ON SEED HARDENING IN SWEET PEPPER

CONFLICTING reports by different workers (quoted by Austin *et al.*<sup>1</sup>) on the usefulness of presowing hardening show that the effectiveness of the treatment depends on the species and moisture content and fertility of the substratum. It was thought worthwhile to inquire whether the conditions under which the treatment was given influenced its efficacy. As no definite information in this regard was available, studies on the influence of light and temperature on seed hardening were undertaken and the findings on "California Wonder" variety of sweet pepper (*Capsicum frutescens*) are reported below.

The seeds obtained from Sheela Seeds Farm, Srinagar, were surface sterilised with 0.1% mercuric chloride, washed thoroughly with distilled water and were subjected to three cycles of hardening (soaking for 3 hr followed by drying for 21 hr) with kinetin and ascorbic acid at 100 mg/l concentration under the following conditions of light and temperature; (a) hardening in laboratory light conditions at  $27 \pm 1^\circ \text{C}$ , (b) hardening in constant darkness at  $25 \pm 1^\circ \text{C}$ , (c) hardening in constant light from a fluorescent lamp at  $27 \pm 1^\circ \text{C}$ , (d) soaking at  $13^\circ \text{C}$  in dark and drying in diffuse laboratory light at  $27 \pm 1^\circ \text{C}$ , (e) soaking at  $40^\circ \text{C}$  in dark and drying in diffuse laboratory light at  $27 \pm 1^\circ \text{C}$ . The treated seeds were sown in garden soil in polythene bags. Unhardened seeds served as control. As it was found in the previous study that hardening with growth regulator was better than with water (Kanchan, 1973)<sup>2</sup> and as the purpose of the present investigation was only to compare the efficacy of growth regulators in hardening under different light and temperature conditions, the effect of hardening with water has not been considered in the present study. The set was replicated five times. The experiment was conducted during June-July 1973, when the temperature varied from a mean minimum of  $20^\circ \text{C}$  to a mean maximum of  $29.9^\circ \text{C}$  and the relative humidity ranged from 49 to 80 per cent. The containers were watered regularly. Per cent emergence and the performance of fortnight old seedlings were noted.

Hardening under constant light than under constant darkness gave much higher per cent emergence though under either of the light conditions ascorbic acid proved more useful than kinetin (Fig. 1). Laboratory light conditions proved better than constant darkness with kinetin whereas with ascorbic acid it was the other way.

In regard to the height of shoots, hardening under constant light conditions produced the best results though the highest shoot dry weight was due to constant darkness with kinetin and laboratory

light conditions with ascorbic acid (Fig. 1) which was obviously due to broader leaves in the former case (Plate 1-b) and broader second pair of leaves in the latter (Plate 2-c). The effects on depth and dry weight of root system seemed to be entirely the result of interaction of growth regulator and

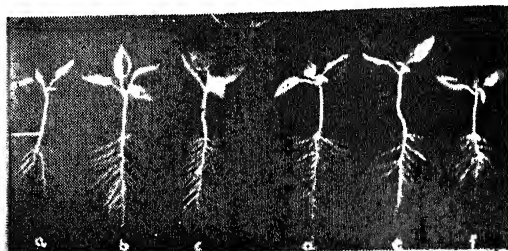
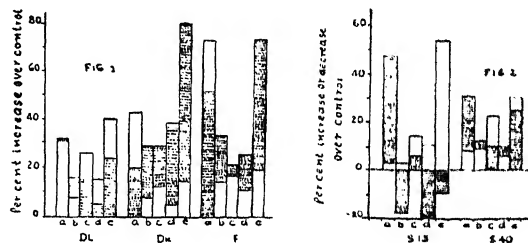


PLATE 1. Showing the effect of seed hardening with kinetin under different light and temperature conditions on seedling growth in sweet pepper.



PLATE 2. Showing the effect of seed hardening with ascorbic acid under different light and temperature condition on seedling growth in sweet pepper. (a) control; (b) hardening in constant darkness at  $25 \pm 1^\circ \text{C}$ ; (c) hardening in constant light (fluorescent lamp) at  $27 \pm 1^\circ \text{C}$ ; (d) soaking in darkness at  $40^\circ \text{C}$  and drying under laboratory conditions; (e) hardening under laboratory light conditions at  $27 \pm 1^\circ \text{C}$ ; (f) soaking in darkness at  $13^\circ \text{C}$  and drying under laboratory light conditions.



FIGS. 1-2. Showing the influence of light and temperature on seed hardening in sweet pepper var. California wonder.

DL-hardening in laboratory conditions at  $27 \pm 1^\circ \text{C}$ , DK-hardening in constant darkness at  $25 \pm 1^\circ \text{C}$ , F-hardening in constant light (fluorescent lamp) at  $27 \pm 1^\circ \text{C}$ . S13-soaking at  $13^\circ \text{C}$  and drying in laboratory conditions, S40-soaking at  $40^\circ \text{C}$  and drying in laboratory conditions.  $\square$ -kinetin treatment,  $\square$ -Ascorbic acid treatment. a-emergence, b-shoot height, c-shoot dry weight, d-root depth, e-root dry weight.

light condition. Hardening with kinetin in the absence of light than in its presence yielded better results whereas with ascorbic acid presence of light was more advantageous (Fig. 1). But on the score of growth regulator alone, kinetin produced much superior and significant results in the presence or absence of light.

Effect of the soaking temperature on emergence depended on the growth regulator used. 13° C was found better than 40° C with kinetin and it was the reverse with ascorbic acid. However at both these temperatures, only kinetin was significantly useful (Fig. 2).

Soaking temperature of 40° C was more useful with kinetin than with ascorbic acid in increasing shoot height. However, ascorbic acid treatment produced broader and more number of leaves and hence greater shoot dry weight (Fig. 2, Plates 1-d and 2-d). On the root system, significant improvement was evident only in dry weight (Fig. 2). Lowering the soaking temperature to 13° C inhibited the linear growth of seedlings (Plate 1-f) and dry weight of root system (Fig. 2) with kinetin treatment and reduced the effectiveness of ascorbic acid treatment on shoot growth. (Plate 2-f) but increased the dry weight of root system (Fig. 2).

The foregoing account makes it obvious that post-hardening manifestations are also influenced by light and temperature conditions prevailing during the treatment. In California wonder variety of sweet pepper, presence of light during hardening improved per cent emergence and linear growth of shoot to a great extent, influencing the action of ascorbic acid more than that of kinetin. But root growth, leaf size and number depended to a greater degree on the interaction of these growth regulators with other light conditions. Thus the possibilities of achieving better results from hardening by manipulating suitably the light conditions during the treatment seem bright. Soaking temperature of 40° C did not offer any advantage over that at 27° C with kinetin and reduced the effectiveness of ascorbic acid treatment significantly. Low soaking temperature (13° C) caused slight improvement in emergence per cent as compared to that at 27° C only with kinetin treatment but this advantage was largely outweighed by distinct inhibition of the linear growth of the seedlings. Ascorbic acid treatment was also less effective at this soaking temperature. Thus hardening with kinetin or ascorbic acid at temperatures much higher or lower than usual laboratory temperature at 27° C did not offer any advantage. It would be interesting to test whether during hardening the light conditions override the influence of temperature. Further studies elaborating these aspects should reveal interesting and useful information.

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October 12, 1973.

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#### POSSIBLE ROLE OF ORGANIC ACIDS IN THE ERGOT DISEASE OF BAJRA (*Pennisetum* *TYPHOIDES* L.)

YOUNG earheads of bajra (*Pennisetum typhoides* L.) were observed to be highly susceptible to the ergot disease [*Claviceps microcephala* (Wallr.) Tul.] while the fertilized earheads were resistant. The resistance developed 8 days after emergence of earheads may be due to the fertilization of the pistil<sup>1</sup>. Since organic acids were found to be poor carbon source for the growth of the fungus *in vitro*, the organic acids content of bajra earheads at different ages was analysed. One gram of fresh spikelets from 1, 2, 4, 8, 12 and 20 days old earheads was ground in a mortar with 80% hot alcohol and centrifuged at 3000 rpm for 15 min. Three volumes of chloroform were added to the supernatant, shaken well and the chloroform layer was discarded. The process was repeated three times. The resultant solution was then evaporated to dryness *in vacuo*. The contents were dissolved in 1 ml of 80% alcohol and known volume of the solution was chromatographed using *n*-butanol-formic acid-water (10:2:15 V/V/V) as solvent system. The spots were detected by spraying 0.4% bromocresol green (adjusted to pH 7.0 with 0.1 N sodium hydroxide). The organic acids were identified by comparison with  $R_f$  values of pure substances. Area of the spots occupied by each organic acid on the chromatogram developed with the same volume of the earhead extract was calculated to get semiquantitative values. Standard curve was prepared by spotting different quantities of malic acid and the results were expressed in  $\mu$ g of malic acid constant per g of fresh earhead.

Seven organic acids were found to be present in the healthy bajra earheads, out of which only four of them were identified. They were maximally present during the disease resistance stage (Table I). Organic acids content of bajra earheads was analysed at different stages of disease development also. The earheads were inoculated with the pathogen on the fourth day of their emergence. Honey dew exudation was observed 4 days after inoculation and heavy exudation was observed 16

TABLE I  
Organic acids content of bajra earheads at different ages

Age of the earhead	Susceptible or resistant	Organic acids in $\mu\text{g/g}$ fresh weight expressed as malic acid constant						
		Tartaric acid	Citric acid	Malic acid	Oxalic acid	Unidentified acid ( $R_f=0.08$ )	Unidentified acid ( $R_f=0.15$ )	Unidentified acid ( $R_f=0.17$ )
1 day	Susceptible	200	640	320	480	200	600	600
2 days	"	440	1200	400	480	400	600	560
4 "	"	600	720	320	480	320	600	320
8 "	"	680	640	240	560	400	600	440
12 "	Resistant	2400	2800	800	720	400	1000	1000
20 "	"	2400	1680	800	600	240	720	820

days after inoculation. Comparable healthy earheads were also analysed as controls. Besides the seven organic acids detected in the healthy earheads, one more organic acid, viz., succinic acid was found in the diseased earheads (Table II).

TABLE II  
Changes in organic acids content of bajra earheads due to ergot infection (expressed as malic acid constant in  $\mu\text{g/g}$  fresh weight)

Organic acid	Honey Dew initial stage		Honey Dew advanced stage		Sclerotial initial stage	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Tartaric acid	680	1080	2400	2400	2400	3000
Citric acid	640	880	2800	600	1680	480
Malic acid	240	560	800	880	800	1200
Oxalic acid	560	560	720	980	600	600
Succinic acid	0	600	0	440	0	680
Unidentified acid ( $R_f=0.08$ )	400	240	400	240	240	240
Unidentified acid ( $R_f=0.15$ )	600	690	600	600	1000	600
Unidentified acid ( $R_f=0.17$ )	320	720	440	680	1000	640

Efficacy of various organic acids (tartaric, succinic, malic, citric and oxalic acids) in supporting the growth of the pathogen *in vitro* was assessed by growing the pathogen in Kirchoff's medium in which carbon source was substituted with the organic acids and adjusted to pH 7.0 with 0.1 N sodium hydroxide. All these organic acids did not support the growth of the pathogen. Hence the accumulation of excess organic acids may be inhibitory to the development of ergot fungus. It was also found that the extra organic acid like succinic acid appeared due to infection. The accumulation of these organic acids might be playing a role in the defensive mechanism which aims at the prevention of disease development.

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#### SOIL TRANSMISSION OF AN AQUATIC SMUT

INFECTION by an aquatic smut *Narasimhanialismatis* Pavgi and Thirumalachar appears on the floating leaves of *Alisma reniforme* D. Don. in roadside ponds during July–September every year in Varanasi, U.P., and the clathroid teliospore balls mature in the leaf tissues in August<sup>2</sup>. The incidence reaches the peak in mid-September covering most of the laminar area embedding mature spore balls. The pond water recedes during October and dries in December, when the infected leaves wither and fall on the ground as debris, gradually decomposing and releasing the spore balls, while the root stocks or rhizomes remain dormant deep in the soil until the following monsoon. Viable inoculum for infection appears to be provided through systemic infection of the rhizomes or the spore balls released in soil from the debris. Fresh infection spots without deformation of leaves and absence of parasitic mycelium in the petioles and rhizomes indicated absence of systemic host infection and the over-summering teliospores alone constituted the primary inoculum. The dormant teliospores from the spore balls germinate during August and the terminally borne sporidia produce on expulsion secondary sporidia which conjugate in compatible pairs to develop dikaryotic hyphae initiating fresh infections.

Soil samples were collected in replicates from the infested pond beds from December through June. One gram soil was suspended in 10 ml sterile dist.

water, the supernatant collected and centrifuged at 1000 rpm. The sediment yielded 50–80 spore balls/gram soil, most of them containing germinable teliospores. This was, however, a high number relative to the spore balls released from the debris alone and represented their addition through saprobic development in the marsh, until the soil moisture and temperature permitted. Artificial cultures from single teliospores and mass sporidia (containing compatible complements) on potato dextrose agar (with 0.5% yeast extract, pH 6.5) and 10% soil extract dextrose agar yielded development of mature teliospore balls containing germinable teliospores compacted in the dermat (Fig. 1). Companion

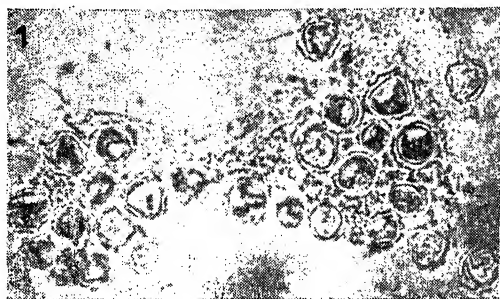


FIG. 1. Portion of clathroid spore ball in artificial culture showing teliospores in immature dermatal reticulum,  $\times 750$ .

cultures on moist pond soil also yielded clathroid spore balls containing viable teliospores, confirming ability for saprobic development by the pathogen. This ability of *N. alismatis* adds source enriching the inoculum potential for the following season. Thermophilic development and heat resistance of the teliospores through the summer months appear characteristic of this genus/species. Species of other aquatic smut genera in the family Tilletiaceae such as *Burrillia* Setchell, *Doassansia* Cornu, *Doassansiopsis* Dietel, *Entorrhiza* Weber and *Tracya* Sydow<sup>1,3</sup> are possibly perpetuated likewise through saprobic teliospore development in the soil.

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## ON SOME SPECIES OF ZYGNEMA FROM JAMMU

*Zygnema* is a very large genus with more than 100 species reported from all over the world. Randhawa<sup>1</sup> lists 23 species as Indian and some more have been added to the existing list. In order to supplement the data on the distribution of *Zygnema* within the country, an effort is being made to collect the species from JAMMU (J. & K.).

The author has so far been able to collect four species of *Zygnema*, from Jammu. Of special mention is *Z. indicum*—a species described by Misra<sup>2</sup> from Kashmir. The validity of this species is doubted by Randhawa<sup>1</sup> as only immature zygospores were reported by Misra. Mature zygospores have now been collected by the author. Lateral conjugation (not seen by Misra) has also been observed, apart from other variations, as pointed out in the text. The variations are attributed to the differences in the climatic and other ecological factors which are quite different in Kashmir and Jammu.

### DISCUSSION

#### 1. *Zygnema czurdae* Randhawa<sup>3</sup>

Free-floating, bluish-green; cells short, cylindrical,  $18.5 - 22.3 \times 76.3 - 89.1 \mu$ ; chloroplasts 2, irregular, somewhat globose; pyrenoid one in each chloroplast; conjugation both lateral and scalariform; zygospores smooth, oval; azygospores not found.

From a pond at Udampur mixed with *Spirogyra*, March 1972. Figs. 1–2.

#### 2. *Zygnema indicum* Misra<sup>2</sup> emend.

Free-floating, green; cells cylindrical, broader and shorter than *Z. czurdae*; vegetative cells  $35.2 - 43.1 \times 52.3 - 70.3 \mu$ ; chloroplasts 2, stellate with conspicuous pyrenoids in the centre of each chloroplast; conjugation both lateral and scalariform; lateral conjugation observed only in some specimens collected from an altitude of 6,500 ft.; zygospores in the conjugation canal or in one of the gametangia; conjugation canals (in scalariform conjugation) inflated; zygospores  $23 - 28 \mu$  long, oval; exospore rough; mesospore smooth and thick, blue in colour.

In a pond at Patnitop and Udampur, April 1972.

Figs. 3–5.

Randhawa<sup>1</sup> considers the species doubtful as only immature zygospores were found by Misra<sup>2</sup>. The author has found mature zygospores in the Patnitop material. Mature zygospores are slightly bluish in colour. The validity of the species need no longer be questioned. In the author's opinion this species

does not resemble any other known species. Hence *Z. indicum* is to be retained as a valid species. As the author has also found lateral conjugation in this species, an emended description is given above.

3. *Zygnema melanosporum* Lagerheim<sup>4</sup>

Free-floating, dark-green; cells 4–5 times as long as broad,  $24.1-30.3 \times 80.2-98.4 \mu$ ; chloroplasts 2, slightly rounded; pyrenoids one in each chloroplast, prominent; conjugation scalariform; zygospores in one of the gametangia, ovoid,  $20-25 \times 28-34 \mu$ ; mesospore blue, punctate.

From freshwater pond at Udhampur. March 1972.

Figs. 6–7.

4. *Zygnema atrocoeruleum* W. & G. S. West<sup>5</sup>

Free-floating, green, mixed with *Z. melanosporum*; Cells very narrow, almost rectangular,  $12.3-16 \times 28.5-47.9 \mu$ ; chloroplasts 2, almost occupying the cell, stellate, pyrenoids 2 placed on the lateral sides of the chloroplasts; conjugation scalariform; zygospores in one of the gametangia; zygospores globose, smooth, blue in colour.

From freshwater pond at Udhampur. March 1972.

Figs. 8–9.



FIGS. 1–9

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A CASE OF RARE TRIPLOIDY IN EXCISED  
ROOT CULTURES OF *TRIGONELLA*  
*FOENUM-GRÆCUM* LINN.

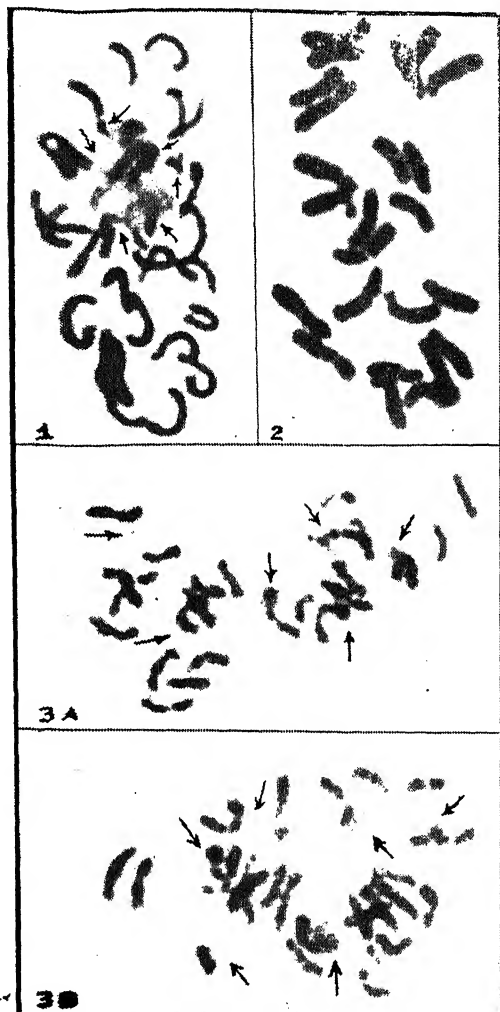
*Trigonella foenum-græcum* Linn., commercially known as fenugreek, has a diploid complement of sixteen chromosomes. Of these, two of the pairs are satellited and form four nucleoli<sup>1</sup>. Induction of polyploidy has been shown to be possible by treatment with colchicine and some *c*-mitotic chemicals<sup>2-4</sup>. There is, however, no report on the spontaneous occurrence of polyploidy in this species.

During an analysis of the behaviour of the chromosomes in young excised roots in culture, occurrence of a triploid was observed in one of the four roots grown in 30 ml of modified White's medium<sup>1</sup> for three days at 30° C. The analysis of the roots was conducted by fixing them in acetic alcohol for 24 hr and postfixation in formaldehyde-acetic alcohol for 15 min and then processing as haematoxylin squashes<sup>5</sup>. A prophase with 24 chromosomes observed therein is illustrated in Fig. 1. Six of the chromosomes appear to be satellited. A single large nucleolus observed may be clearly seen among the chromosomes depicted in this figure. In Fig. 2, the twenty-four chromosomes encountered can be easily counted. The two early telophasic groups, presented in Fig. 3 A and B, reveal in each a complement of six nucleoli. The availability of only one triploid, that too accidentally, precluded a detailed analysis of the behaviour of the chromosomes in this triploid.

The occurrence of gametes with an unreduced chromosome number is said to be common in dicotyledons<sup>6</sup>. Their fusion with gametes having a reduced chromosome complement would ordinarily result in the appearance of triploids. It is reasonable to presume therefore that the triploid *Trigonella foenum-græcum* should have originated in this manner. The chromosome number, in fact, could easily be ascertained, besides making it possible to elucidate the number of satellites and nucleoli



originating therefrom. Indeed, it was possible to count six SAT-grains at prophase (Fig. 1). Thus, the presence of six nucleoli at early telophase tends to confirm that each of them should have originated in association with a SAT-chromosome.



FIGS. 1-3. Fig. 1. Prophase with 24 chromosomes. Note 6 satellites (arrows) and a large nucleolus,  $\times$  ca. 3,800. Fig. 2. Metaphase with 24 chromosomes,  $\times$  ca. 3,900. Fig. 3 A and B. Two groups of chromosomes from a telophase. Note six nucleoli in each (arrows),  $\times$  ca. 5,200.

The author is indebted to Dr. M. K. Subramaniam and Dr. (Mrs.) Saraswathy Subramaniam for advice and to Prof. J. V. Bhat, Prof. H. Shantaram and Dr. A. Krishna Rao, Dean, for encouragement.

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### A NEW SPECIES OF *DENDRYPHIELLA*

IN this communication the authors propose to describe a new species of *Dendryphiella* Bub. & Ranoj collected by them during their studies on Hyphomycetes.

*Dendryphiella indica* Rao & Naranian sp. nov.

Colonies effused, reddish-brown, spreading 1-3 mm. Mycelium subhyaline to pale brown, septate, branched, 1.8-3.6  $\mu$  wide. Conidiophores erect dichotomously or sympodially or irregularly branched, macronematous, mononematous, 150.0-540.0  $\mu$  long, 3.6-5.0  $\mu$  broad at base, 3.6-4.2  $\mu$  broad at apex, smooth or verrucose, thick-walled and septate. Conidiogenous cells polytretic, oval, subspherical or clavate, cicatrized 30.0-55.0  $\mu$  long, 3.6-4.2  $\mu$  broad at base and 10.0-15.0  $\mu$  broad at apex with 3-7 scars, dark blackish-brown, thick-walled. Conidia tretic, solitary or catenate, acropleurogenous, dry, cylindrical or oblong, rounded at both ends, 1-3 septate, pale to dark brown, 15.0-30.0  $\mu$  long, 5.0-9.0  $\mu$  broad, tuberculate.

Type collection in Herb. V.V.C.B.L. No. 1301 on dead leaves of *Aloe* spp. from Nehru Zool. Park, Hyd., 20-11-1973, V.R.

*Dendryphiella indica* Rao & Naranian sp. nov.

Colonies effusae rufus-brunneous 1-3 mm metients. Mycelium ramosis, hyphis septatis, subhyalinis vel pallide brunneis 1.8-3.6  $\mu$  crasis. Conidiophoras dispersa, recta vel curvata, ramosae dichotomous, sympodia vel irregularibus, macronematous, mononematous, 150.0-540.0  $\mu$  longa, 3.6-5.0  $\mu$  basim. 3.6-4.2  $\mu$  ad apicem, laevis ad verrucatus, pachydermicus, septatae. Conidiogencellulae polytretic, ovalis, subsphaericos, vel clavatus, cicatricibus 30.0-55.0  $\mu$  longa, 3.6-4.2  $\mu$  lata ad basim et 10.0-15.0  $\mu$  lata ad apicem cum 3-7 cicatrices (poris) fuscibrunnei, pachydermis. Conidia poris (treticus) solitarius vel catenulas, acropleurogenae cylindriceae vel oblonga, rotandus basim ad apicem, 1-3 septatis pallidus vel





for symptom development with the lower concentration.



FIG. 1. Hypersensitive reactions induced in the cotyledonary leaves after 72 hr of infiltration with *Xanthomonas oryzae* at  $10^8$  cells/ml. (A) cotton, left: control (water infiltrated), right: bacteria infiltrated. (B) okra, left: control (water infiltrated), right: bacteria infiltrated.

A large number of strains of *X. oryzae* collected from different parts of India were tested for induction of HR in cotton and okra. All the strains gave positive reactions in both the plants whereas some of the commonly encountered yellow saprophytes on rice leaves were found to be negative.

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#### STUDIES ON A VIRUS CAUSING MOSAIC DISEASE IN BOUGAINVILLEA

BOUGAINVILLEA (*Bougainvillea glabra* Choisy var. *Sanderiana*, Hort.) is an important ornamental plant popularly grown in public and private gardens. In a survey of virus diseases of ornamental plants, *Bougainvillea* was found to be affected by a mosaic disease in some gardens of Gorakhpur. Preliminary tests showed that the causal organism was a virus. The leaves of affected plants showed mosaic mottling. The size of the leaves of severely affected plants was slightly reduced and distorted (Fig. 1). Diseased plants showed retarded growth with small flowers. The number of flowers produced were much less in number when compared to healthy ones.

The disease was mechanically transmitted to a number of plants which includes *Bougainvillea glabra*, *Capsicum annuum* L., *Cucumis sativus* L., *Cucurbita pepo* L., *Datura metel* L., *D. stramonium* L., *Melilotus alba* Medicus, *Nicotiana tabacum* L. var. White Burley, *Petunia hybrida* Vilm., *Physalis peruviana* L., *Salvia plebeia* R. Br., *S. splendens* Ker. Ganl., *Solanum melongena* L., *S. nigrum* L.,

*Trifolium repens* L., *Vigna sinensis* Savi. *Vinca rosea* L. and *Zinnia elegans* Jacq.

The virus was inactivated at a dilution of 1: 10,000 and temperature between 65–70°C. The

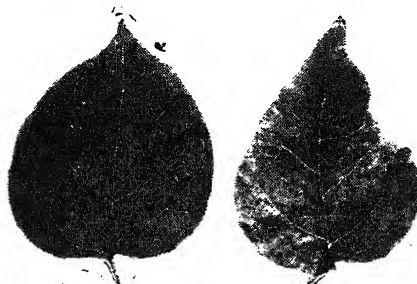


FIG. 1. Leaves of *Bougainvillea glabra*. Left-Healthy; Right-Diseased.

longevity *in vitro* was 2–3 days at room temperature (30–35°C). The virus was easily transmitted by *Myzus persicae* Sulz. and *Aphis gossypii* Glov. and was found to be of non-persistent type.

From Florida Bestagno<sup>1</sup> described a ring spot type virus occurring in *Bougainvillea*. The other record of a mosaic disease is by Ganga<sup>2</sup>. The observations made on the virus disease of *Bougainvillea* in the present studies show that the causal agent has a host range, physical properties, insect vectors similar to that of cucumber mosaic virus as reported by Smith<sup>3</sup>. Cucumber mosaic virus has been recorded widely on the economic plants from this area (Joshi and Dubey<sup>4,5</sup>, Dubey<sup>6</sup>). The presence of this virus on a perennial host like *Bougainvillea* will act as a potential source of infection to different economic plants throughout the year. Hence, it is suggested that diseased plants of *Bougainvillea* be eradicated to avoid the further spread of cucumber mosaic virus in nature.

Our sincere thanks are due to Prof. K. S. Bhargava for providing necessary facilities for the work. We express our indebtedness to the U.G.C. for financial assistance.

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February 7, 1974.

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### *JATROPHA GOSSYPIFOLIA*, A NEW HOST OF SOME PATHOGENIC FUNGI

*Jatropha gossypifolia* L. (Euphorbiaceae), a native of Brazil, has now become naturalised in some parts of India. During the course of investigation on leaf-pathogenic fungi of Meerut and its neighbourhood, severe spots on leaves of *J. gossypifolia*, growing in the Meerut College, Meerut Campus, were observed in the month of November, 1973. Diseased leaves were collected for detailed studies on the causal organism(s). Microscopic examination of the transverse sections of leaves through spots and of the preparations from such spots of various leaves revealed the presence of three pathogenic fungi, none of which has so far been reported on this host. Since all these fungi are new host records, their morphologic characters are being given below.

1. *Cercospora* leaf spot.—Leaf spots circular when young becoming irregular to angular at maturity, amphigenous, pale yellow to brown, scattered to coalescing, variable in size, stromata of few brown cells; conidiophores in fascicles, cylindrical, geniculate above, pale yellow to brown, simple, continuous, a few 1–2 sparingly septate; conidia hyaline with slight olivaceous tinge, mostly acicular a few obclavate, straight or slightly curved, attached by the larger blunt end, 3–12 septate, not constricted at septa (CH, MCM 202).

On the basis of detailed morphologic characters and measurements of conidiophores and conidia, the fungus was identified as *Cercospora euphorbicola* Atk. From India, this fungus has so far been reported only on *Euphorbia* sp. by Chona *et al.*<sup>1</sup> and thus the present report of the fungus is second from the country.

2. *Helminthosporium* leaf spot.—Leaf spots brown, irregular; conidiophores olivaceous brown, simple, septate; conidia obclavate to cylindrical, olivaceous brown. 3–7 septate (CH, MCM 203).

The fungus was successfully isolated from diseased host leaves on P.D.A. and the pathogenicity

was established by inoculating the healthy leaves with the spore *cum* mycelial suspension produced in culture. On the basis of detailed morphologic characters the fungus was identified as *Helminthosporium euphorbiae* Hansf., the other two reports of which from the country are those of Rao and Kelkar<sup>2</sup> and Sohi *et al.*<sup>3</sup> on *Euphorbia geniculata* and *E. pulcherrima* respectively.

3. *Colletotrichum* leaf spot.—Comparatively very few leaves showed infection by this fungus. Leaf spots amphigenous, more or less circular, mostly confined to the margins of lamina, 4–8 mm diam., ash-coloured with dark centre; acervuli epiphyllous, few, scattered, subepidermal, erumpent, more or less hemispherical; setae dark, septate, rigid, straight, pointed; conidiophores hyaline, short, arranged in a palisade manner; conidia hyaline, one-celled, mostly cylindric, a few cylindric-lunate, pointed towards the ends (CH, MCM 204).

The fungus was successfully isolated from diseased leaves on Oat meal medium and the pathogenicity was established by inoculating the healthy leaves with spore inoculum produced in culture. On the basis of its detailed morphologic characters the causal organism was identified as *Colletotrichum gloeosporioides* Penz.

The herbarium specimens and materials of all these fungi have been deposited in the Cryptogamic Herbarium, School of Plant Morphology, Meerut College, Meerut.

Thanks are due to Dr. V. Singh for facilities and encouragement.

Department of Botany,  
Meerut College, Meerut, India,  
May 18, 1974.

P. D. SHARMA.

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## SHORT SCIENTIFIC NOTES

### Computer Program for Conducting Hybrid Analysis from a Line $\times$ Tester Mating System

The line  $\times$  tester analysis is basically a type of genetic analysis which tests the combining abilities (general and specific) of a number of given lines in the genetic background of a number of given or proven testers. This type of analysis provides the basic genetic data with reference to the type of gene action involved in controlling a quantitative character and obviously is very much needed in determining the most productive crosses from a number of available crosses in a hybrid breeding program.

Kempthorne (1957) initially developed a statistical procedure for performing combining ability analysis from a line  $\times$  tester mating scheme. The analysis partitions the total genotypic variation into variation due to lines, due to testers and that due to the interaction between lines and testers. Further it splits the total genotypic variance into variance due to general combining ability (gca) and that due to specific combining ability (sca). The general combining ability effects of lines and testers and specific combining ability effects of the hybrid combinations are also estimated. This type of information is usually obtained from hand calculators by the students which often poses limitations on the number of crosses to be handled as also the time taken in obtaining the information.

We have developed and documented a computer program which performs the hybrid analysis from a line  $\times$  tester mating system for the Indian made T.D.C.-12 computer. The program is written in Fortran 4-K-language and could be utilized for obtaining the relevant information from the above-mentioned computer. It handled 225  $F_1$  combinations resulting from a  $15 \times 15$ , line  $\times$  tester mating scheme, grown in a randomized block design with 4 replications.

The computer output gives the following informations :

1. Specific combining ability effects of  $F_1$  combinations.
2. General combining ability effects of lines and testers.
3. Analysis of variance table partitioning all the source of variation into components as described by Kempthorne (1957).

4. Analysis of genotypic variance into variance due to general combining ability and variance due to specific combining ability.

Details of the program along with a worked example could be obtained from us.

Thanks are due to Drs. N. K. Anant Rao, K. G. Gollakota and R. L. Paliwal for encouragement and facilities. One of us (K. V. Peter) acknowledges ICAR, New Delhi, for the award of a Senior Fellowship.

Dept. of Plant Breeding,	K. V. PETER.
Dept. of Mathematics and	B. RAI.
Statistics,	V. K. SRIVASTAVA.
G.B. Pant Univ. of Agri.	R. C. JAIN.
and Technology,	
Pantnagar (Nainital), May 23, 1974.	

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### Detection of Ragi (*Eleusine coracana*) in Mustard Seeds (*Brassica nigra*)

Due to similarity in physical appearance ragi and Argimone (*Argimone mexicana* Linn.) seeds are used as adulterants in mustard seeds. A method using paper chromatography has been reported for the detection of Argimone seeds in mustard seeds<sup>1</sup>. No chemical method is available for the detection of ragi seeds in mustard seeds. The present study describes a chemical method for the detection of ragi in mustard seeds based on our observation that ragi contains amylose which gives blue colour with iodine whereas mustard and Argimone seeds do not.

*The test.*—Few local varieties of ragi and mustard seeds were purchased from government agencies. Argimone seeds were procured from the Department of Agriculture, Mysore, Bangalore. The seeds were freed from foreign matters, powdered separately in the grinder attachment of waring blender and the fine powder was used for the study. Two grammes of each of the powdered ragi, mustard Argimone and 2 g mustard containing different percentages of ragi were taken in separate test-tubes. The samples were boiled for 5 minutes with 15 ml of water, cooled and filtered. Few drops of iodine

solution (0.2% iodine in 2% potassium iodide solution) were added to each tube and shaken well. Blue colour characteristic of amylose-iodine complex was observed in ragi and mustard containing ragi at 0.1% level of adulteration. No blue colour was observed in the case of Argimone and mustard seeds.

Amylose content in different varieties of ragi was determined by the colorimetric method of McCready *et al.*<sup>2</sup>.

No significant variation in the concentration of amylose was observed among different varieties.

The test is sensitive and adulteration of mustard with ragi as low as 0.1% can conveniently be detected by this method.

Food and Water                      N. G. DIVAKAR.  
Analysis Laboratory.              U. N. NAGARAJA RAO.  
Anandaram Circle.                C. P. HARTMAN.  
Bangalore-560009. October 30, 1973.

1. Hartman, C. P., Nagaraja Rao, U. N. and Divakar, N. G., *Curr. Sci.*, 1972, 41(12), 465.
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#### A New Leaf Spot Disease of Mulberry Caused by *Fusarium concolor* Reinking

A severe leaf spot disease of Mulberry (*Morus alba* L.) was observed during the winter season of 1972 and in the subsequent year near Madanapalle in Chittoor District. The disease was manifested by numerous, scattered sharply defined brownish necrotic spots of 1-3 mm diameter, mostly on the lower leaves of the plant. The affected leaves ultimately become chlorotic, resulting in early senescence and leaf fall.

Pure culture of the fungus was obtained by usual plating on PDA medium and single spore culture technique. Pathogenicity was proved by spraying the leaves of one month old plants with spore suspension made in sterile water from two weeks old culture grown on PDA medium and keeping the inoculated plants in a humid chamber. The typical symptoms developed after 4-5 days of inoculation. Reisolations were made and each time the original fungus could be obtained from the artificially infected leaves.

Mycelium dull white, fatted with dark orange coloured pigment developed on the surface of the

medium after 2-3 days. Chlamydospores prominent, globose, smooth walled 10-15  $\mu$  in diameter, both terminal and intercalary in the hyphae, mostly in chains. Macroconidia abundant, 4-6 septate, slightly sickle-shaped tapering at both the ends and measure: 12-16  $\times$  0.3-0.6  $\mu$  (4 septate); 15-24  $\times$  1.5-3  $\mu$  (6 septate). Microconidia are not prominent, but formed very sparsely in old cultures measuring 1-3  $\times$  0.2-0.5  $\mu$ . A culture sent to CMI, England, has been identified as *Fusarium concolor* Reinking (IMI, 183272).

Thanks are due to Dr. C. Booth, Assistant Director, CMI, Kew, England, for identification of the fungus and to Prof. V. S. R. Das for providing the facilities.

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A.U.P.G. Centre,  
Guntur-5, June 4, 1974.

## ANNOUNCEMENTS

### XXV International Congress of Pure and Applied Chemistry

The 25th IUPAC Congress will be held in Jerusalem under the sponsorship of the Israel Academy of Sciences, the Hebrew University of Jerusalem and the Israel National Council of Research and Development, during 6-11 July 1975. The five major areas to be covered in the Congress are (A) Organic Chemistry, (B) Physical Chemistry, (C) Medicinal Chemistry, (D) Applied Chemistry and (E) Macromolecular Chemistry. Further information can be had from the Organising Committee, the 25th Congress of the IUPAC, P.O.B. 16271, Tel Aviv, Israel.

### Award of Research Degrees

Berhampur University, Berhampur, has awarded the Ph.D. degree in Chemistry to (Miss) Susila Devi for her thesis entitled "Structure and Reactivity in Oxidation by Transition Metals".

The M.S. University of Baroda, Baroda, has awarded the Ph.D. degree in Physics to Shri Girishbabu Ravashankar Pandya for his thesis entitled "Study of Crystal Surfaces—(Bi-Sb alloys)"; in Zoology to Shri Menon K. Gopinath for his thesis entitled

University, Hyderabad, for his thesis entitled "Metal Ion Catalysis of the Hydrolysis of Methyl Acetoacetate by the Enzyme, Acetoacetylase". He has also been awarded the Ph.D. degree by the University of Hyderabad for his thesis entitled "Studies on the Kinetics of the Hydrolysis of Methyl Acetoacetate by the Enzyme, Acetoacetylase". He is currently working as a Lecturer in Chemistry, University of Hyderabad, Hyderabad.

Dr. G. V. Subrahmanyam, Professor of Chemistry, University of Hyderabad, Hyderabad, has been awarded the Ph.D. degree by the University of Hyderabad for his thesis entitled "Studies on the Kinetics of the Hydrolysis of Methyl Acetoacetate by the Enzyme, Acetoacetylase". He is currently working as a Lecturer in Chemistry, University of Hyderabad, Hyderabad.

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He is currently working as a Lecturer in Chemistry, University of Hyderabad, Hyderabad.

University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Smt. Saughamita Biswas, for her thesis entitled "Complexes of Serpentine with Chlorides and Nitrates of Potassium and Uranium".

S. Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri P. Venkataramana for his thesis entitled "Studies in Heterogeneous Equilibria: Surface Area Measurement".

S. Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri D. Venkata Reddy for his thesis entitled "The Studies in Reduction Reaction in Analytical Chemistry: Ammonium Thiocyanate as Reducing Agent".

---

## REVIEWS AND NOTICES OF BOOKS

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**General Entomology.** Second Edition. By M. S. Mani. (Oxford and IBH Publishing Co., 66 Janpath, New Delhi-1), 1973. Pp. xiii + 597. Price Rs. 18.75.

The present second enlarged edition of the book contains not only about 100 pages more than the original edition but also the enlarged chapters are reorganised. Thirty-one chapters included in the book deal with the different aspects of general entomology such as structure, function, embryology, distribution and classification.

The first ten chapters are devoted to the structure and function of different systems of insects. Over 30 extra pages included in this section over that of the first edition provide more and up-to-date information on several aspects of functions in insects. An account of the embryonic development and of the different kinds of metamorphosis exhibited by insects during their post-embryonic development is furnished in Chapter XI. Chapter XII is devoted to the influence of the different abiotic factors on insect life, the inter and intra-specific relations and the fluctuations in populations of insects. A good account of geographical distribution of insects giving the characteristic feature of insects and the groups of insects inhabiting different geographical regions of the world is provided in Chapter XIII. Chapter XIV gives brief account of the fossil insects in the world.

The remaining 17 chapters are devoted to the classification of insects and an account of different orders of insects. While giving a brief account of the history of classification of the insects, the author furnishes an account of the different theories explaining the origin of insects and finally follows 33 order system while giving a key to these orders. All these orders are treated fairly extensively in the remaining chapters. Brief accounts of important

families are furnished with appropriate diagrams of whole insects and of structures of taxonomic importance. Keys for separating different families under each order are provided. The book ends with a useful index of general and technical words.

Careful proof reading would have avoided the printers devil appearing here and there in the text.

This edition being an improvement over the previous edition serves as a valuable text book to students of entomology at the degree level. Certain parts of the book serve as reference material to even postgraduate students of entomology. For a book like this the cost is nominal and as such entomology students should be able to go in for this book which serves as a valuable source of information on general entomology.

G. P. C.

---

### Books Received

*Evolution of the Genus Homo.* By William Howells. (Addison-Wesley Publishing Co., Inc., Reading, Massachusetts, 01867), 1973. Pp. 188. Price not given.

*Calculus of Several Variables.* By Serge Lang. (Addison-Wesley Publishing Co., Reading, Massachusetts, 01867), 1973. Pp. viii + 376. Price not given.

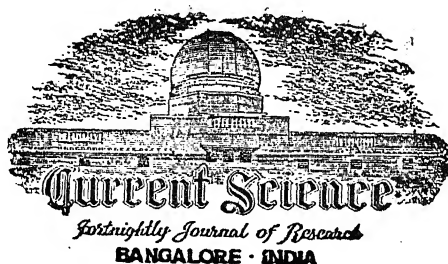
*Mechanisms of Inorganic Reactions—A Study of Metal Complexes in Solution* (Second Edition). By Fred Basolo and Ralph G. Pearson (Wiley Eastern Private Ltd., Pub. J-41 South Extension-1, New Delhi 110049), 1973. Pp. 701. Price Rs. 40.00.

*Physical Anthropology and Its Extending Horizons.* Edited by Amita Basu, A. K. Ghosh, S. K. Biswas and R. Ghosh. (Orient Longmans, Ltd., 3/5 Asaf Ali Road, New Delhi-1), 1973. Pp. 234. Price Rs. 60.00.

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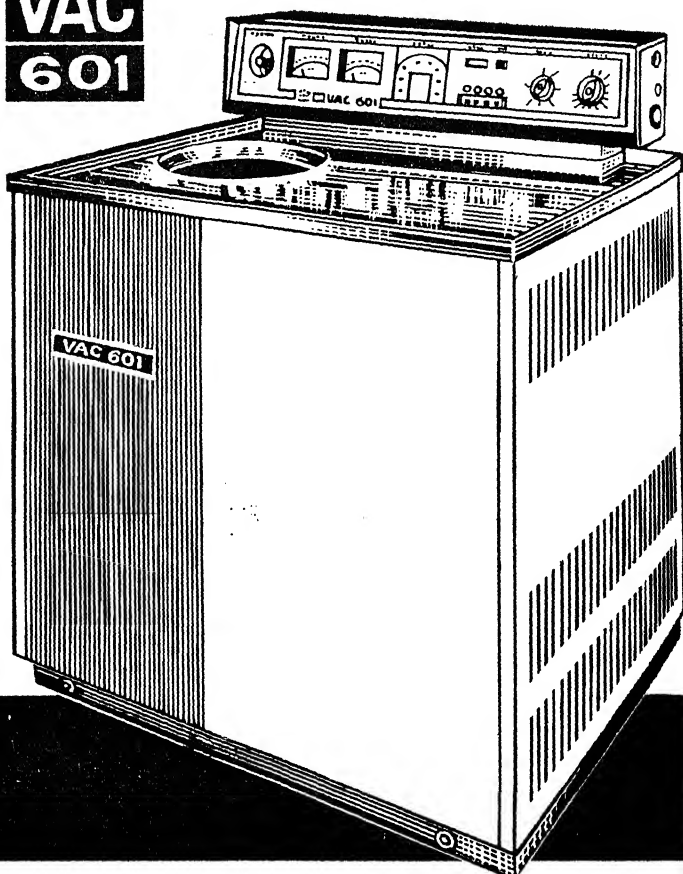


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JAISONS

# DIFFUSE TYPE AND BLANKETING TYPE SPORADIC E AT KODAIKANAL

H. CHANDRA AND R. G. RASTOGI

Physical Research Laboratory, Ahmedabad 380009 (India)

## ABSTRACT

Ionograms at the equatorial station, Kodaikanal, are examined for a period of a solar cycle to study the occurrence of different types of sporadic E, viz., the  $q$  type, the blanketing type, 'no- $E_s$ ' and night  $E_s$ . The daily, seasonal and solar cycle variations in the occurrence of different events at Kodaikanal are compared with the similar occurrence reported at African and American zones. Counter electrojet events greatly control the occurrence of different types of daytime  $E_s$  events.

## INTRODUCTION

**T**OGETHER with the establishment of the rocket launching facility a number of ground based ionospheric experiments were set up at Thumba (dip  $0.6^\circ$  S), near magnetic equator in India to understand the physics of the equatorial ionosphere. The general features of the equatorial ionosphere based on the study of ionograms have been reported (Chandra and Rastogi, 1972 *a, b*). Due to rather small antenna and insufficient overall sensitivity of the ionosonde equipment in the initial stages the sporadic E at Thumba could not be critically examined. To highlight the features of equatorial E-region, ionograms at the neighbouring station, Kodaikanal (dip  $3.4^\circ$  N), were examined for a solar cycle period and the results compared with the ground magnetic and drift (closely spaced receiver method) data. The E-region in the equatorial ionograms is masked by intense sporadic E reflections during the daytime. Rangarajan (1954) has described that there are broadly two types of sporadic E at Kodaikanal.

(1) *Equatorial type*.—Denoted as  $E_{s-q}$ , is the most common type of sporadic E occurring very regularly during daytime and is characterised by its transparency to radio waves. The ground magnetic data shows presence of strong eastward electrojet at times of equatorial  $E_{s-q}$  and drift of electrons as measured by closely spaced receiver technique or backscatter technique is westward (Rastogi *et al.*, 1971; Rastogi, 1973).

We have pointed out a number of occasions when the  $E_{s-q}$  disappears and then the magnetic field value ( $H$ ) drops below its mean night level and the drift of electrons is eastward, (Rastogi, 1972; Chandra and Rastogi, 1973). Such occasions are denoted by symbol 'G' in the publication of ionospheric data. Most of these counter-electrojet events occur in the afternoon hours and are more frequent during low sunspot years than during high sunspot years (Rastogi, 1974).

(2) *Blanketing  $E_s$*  known as  $E_{s-b}$ .—This type of sporadic E usually occurs in the afternoon hours and is most common during J-months in the Indian zone (Bhargava and Subrahmanyam, 1964). The

ground magnetic data show a very weak or reversed electrojet when this type of  $E_s$  occurs and the drift of electrons is predominantly from north to south direction. Equatorward convection of the  $E_s$  layers, from regions where wind shear mechanism can operate, due to Meridional wind is suggested as a possible mechanism to explain  $E_{s-b}$  in equatorial region (Chandra and Rastogi, 1974).

Night  $E_s$  is usually flat and low blanketing type in nature and no marked feature has been observed so far in the ground magnetic data at times of night  $E_s$ . The drift of electrons is in general eastward (Misra and Rastogi, 1971). In the present paper we report occurrence statistics of different types of  $E_s$  at Kodaikanal over a solar cycle period.

## Occurrence of Daytime Sporadic E at Kodaikanal

The percentage occurrence of events during daytime hours, i.e.,  $E_{s-q}$ ,  $E_{s-b}$  and 'no- $E_s$ ' plotted half hourly for the months June-July are shown in Fig. 1. The curves shown by dashed line represent high sunspot period and by full-line represent the low sunspot period. Figure 2 shows number of days  $E_{s-b}$  occurred each month and total number of half hourly  $E_{s-q}$  disappearance (07–17 hr) for each month. The salient features observed are:

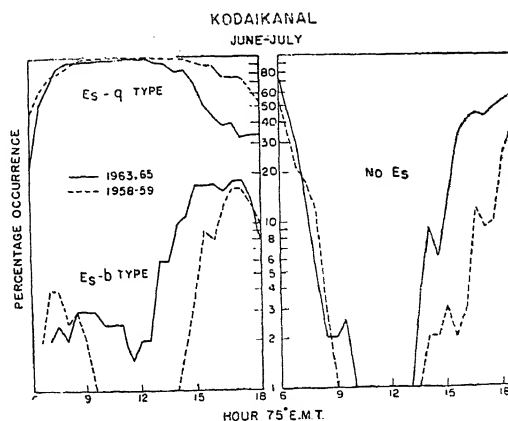


Fig. 1. Daily variations of the percentage occurrence of  $E_{s-q}$ ,  $E_{s-b}$  and 'no- $E_s$ ' during daytime at Kodaikanal for the months June-July of high and low sunspot years.

(a) occurrence of  $q$  type of  $E_s$  is more frequent during high sunspot years than during low sunspot years with maximum occurrence at midday when it is almost hundred per cent. The  $E_{s-q}$  occurrence in the afternoon hours is significantly less during low sunspot year (40% at 1600 hr).

(b) occurrence of blanketing  $E_s$ . It is more frequent during low sunspot years than during high sunspot years. It shows two peaks in the daily variation, one in morning (3%) and another in the evening (15–18%) with minimum at noon (less than 2% for low sunspots and nil for high

sunspots).  $E_{s-b}$  occurrence is maximum during J-months (June–July).

(c) events when 'no- $E_s$ ' occurs are more frequent during low sunspot years than during high sunspot years. Its occurrence is nil at noon but quite significant in the afternoon hours (40% at 1600 hr of low sunspot years). Maximum cases of 'no- $E_s$ ' are observed during D-months.

#### Occurrence of Night $E_s$ at Kodaikanal

Figure 3 describes the percentage occurrence of night  $E_s$  for different months during high, medium and low sunspot years. The nocturnal variations for each season during different periods of solar activity are shown in Fig. 4. The important results are:

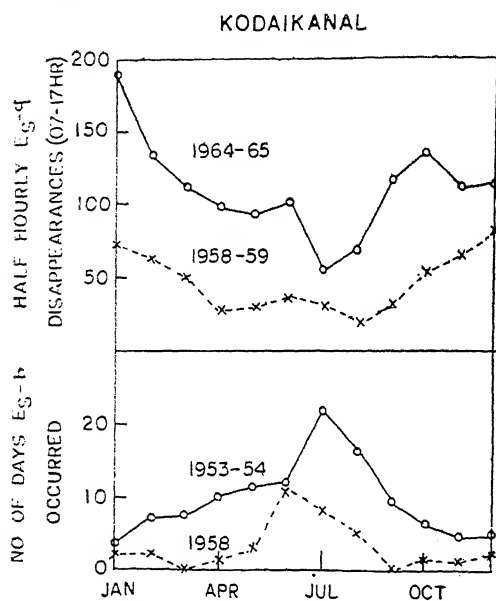


FIG. 2. Occurrence of  $E_{s-q}$  and  $E_{s-b}$  at Kodaikanal during different months of high and low sunspot years.

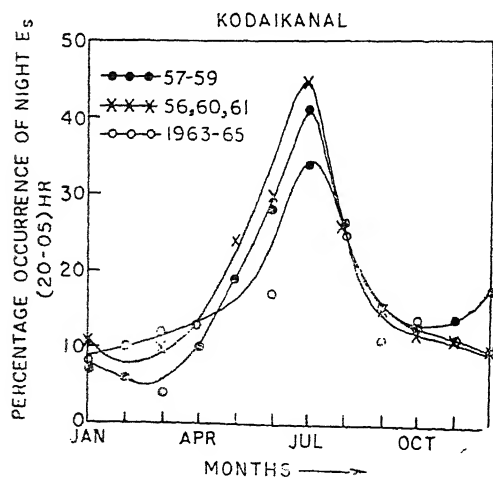


FIG. 3. Percentage occurrence of night  $E_s$  (20–05 hr) at Kodaikanal during different months of high, medium and low sunspot years.

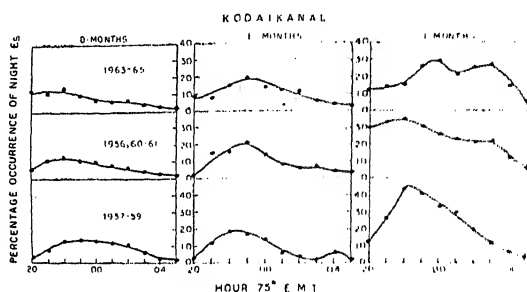


FIG. 4. Nocturnal variations of the percentage occurrence of night  $E_s$  at Kodaikanal during different seasons of the high, medium and low sunspot years.

(i) There is no significant effect of solar activity in the occurrence of night  $E_s$ . For each of the sunspot period, maximum occurrence is noted during J-months (July).

(ii) The peak occurrence of night  $E_s$  is obtained around 22–23 hr.

*Comparison with the results at other equatorial stations.*—A detailed study of the occurrence frequency of different types of  $E_s$  at Huancayo for the IGY was described by Bandyopadhyay and Montes (1963). The non-equatorial type, flat  $E_s$  layers, classified as  $E_{s-f}$  during nighttime and  $E_{s-l}$  (low type) during daytime were studied along with the  $q$  type. The events of 'no- $E_s$ ' during daytime were studied by them for IGY and year 1961–62. Oyinloye (1969) has described occurrence of  $q$  type and daytime blanketing type  $E_s$  at Ibadan for the period 1958–64 and for Zaria for the year 1965. From the results obtained at different equatorial stations one can summarize following points:

(a)  $E_{s-q}$ —Occurrence increases with sunspot number with seasonal maximum in equinoxes (except at Ibadan). The daily variation shows a broad peak at noon, when occurrence is near hundred per cent at Huancayo, Zaria and Kodaikanal but significantly less at Ibadan. The 'no- $E_s$ ' events

known as disappearance of  $E_{s-a}$  are more frequent during low sunspot years than during high sunspot years. While at Kodaikanal its frequency is maximum in the afternoon hours, at Huancayo it is more common in morning hours during E-months and J-months and in afternoon hours during D-months.

(b) *Blanketing  $E_s$* .—The results at Ibadan and Kodaikanal for blanketing type of  $E_s$  are similar which show minor morning peak and major evening peak (16–17 hr), maximum occurrence in J-months and increased occurrence with decreasing sunspot number. The percentage occurrence of evening peak at Kodaikanal (20%) is much higher than that obtained at Ibadan (5–10%). Oyinloye (1971) examined IGY data for the blanketing  $E_s$  ( $f_oE_s - f_oE \geq 0.5-1.0$  MHz) and reported absence of morning peak both at Huancayo and Ibadan. He also reported maximum occurrence during J-months at both the stations; the occurrence being more at Ibadan (6.2%) than that at Huancayo (2.8%). The daily variations at the two stations showed a tendency for afternoon peak which was much clearer for Ibadan. Recently, Kelleher and Kasenally (1972) have reported that occurrence of blanketing  $E_s$  ( $f_oE_s > 5.0$  MHz) at the magnetic equator in the American zone being higher during D-months than during J-months, and an opposite trend at the equatorial stations in the African and far Asian zones. From the above results it seems blanketing  $E_s$  is most common in the Indian zone and least common in the American zone.

(c) *Night  $E_s$* .—The  $E_{s-f}$  occurrence of Huancayo during the IGY was maximum in D-months with postmidnight peak (60%), and minimum during J-months (20%) with premidnight peak. At Kodaikanal the occurrence is maximum in J-months (45%) and minimum during D-months (10%), the peak occurrence is noticed around 22–23 in general for each season. Thus the occurrence of night  $E_s$  is more common in the American zone than in the Indian zone.

#### DISCUSSIONS

The occurrence patterns of the different types of sporadic E during daytime at Kodaikanal are explainable in terms of the occurrence pattern of counter-electrojet currents. It has been shown that at times of normal electrojet  $E_{s-q}$  occurs, at times of counter-electrojet there is no  $E_s$  and at times of very weak (normal or reversed) electrojet with equatorward wind  $E_{s-b}$  occurs. Further the seasonal variation of the blanketing  $E_s$  outside the equatorial region shows maximum during J-months (in the Indian stations). In a similar fashion one can explain occurrence of similar events in the African and American zones.

Rastogi (1974) has shown that the counter-electrojet events occur mainly in the afternoon

hours at Kodaikanal during all seasons. But at Huancayo the occurrence is comparable in the morning and afternoon hours during E- and J-months and mainly in the afternoon hours during D-months. The occurrence of counter-electrojet events at Kodaikanal is much more frequent than at Huancayo.

The occurrence of night  $E_s$  is independent of solar cycle and therefore needs entirely different explanation. Rocket measurements at Thumba during nighttime have shown existence of large-scale structure and presence of valley in the electron density profiles; the presence of irregularities was located mainly in the regions of negative density gradients (Prakash *et al.*, 1970). Presence of eastward electron drift at times of night  $E_s$  favours the nighttime irregularities at the altitudes of negative electron density gradients and caused by cross-field instability.

Beer and Moorcroft (1972) have suggested concept of a combined effect of ionization movements due to the wind shear mechanism and due to the cross-field gradient drifts and explained qualitatively the observed nighttime electron density profiles, and some of the features of the constant height type night  $E_s$ . This concept works efficiently for westward directed background electric field hence predominantly a nighttime phenomenon. Question remains then how to explain the seasonal dependence, *i.e.*, J-maximum in Indian zone and D-maximum in the American zone.

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## SHOOT-BUD DIFFERENTIATION IN STEM-CALLUS TISSUE OF *CITRUS GRANDIS* AND CORRELATED CHANGES IN ITS FREE AMINO ACID CONTENT

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### ABSTRACT

Stem-callus tissue of *Citrus grandis*, free from shoot-buds (undifferentiated tissue), produced numerous shoot-buds (differentiated tissue) in the medium supplemented with 0.25 mg/l BAP + 0.1 mg/l NAA. Undifferentiated and differentiated tissue showed quantitative as well as qualitative differences in their free amino acid content. In general, the amount of free amino acids increased considerably in the differentiated tissue.

It is well known that under the influence of cytokinins, or a combination of cytokinin and auxin, many plant tissues in culture may be induced to differentiate organized structures<sup>1-2</sup>. However, nothing is precisely known about the intervening biochemical changes taking place in the cells of the cultured tissue, between the application of the phytohormones and organogenesis. Qualitative and quantitative changes in respect of particular proteins (enzymes) might play decisive role in morphogenesis. Hence, it may also be worthwhile to study the amino acid pool of the cultured tissue, in relation to the formation of organized structures. Qualitative analysis of free amino acids in undifferentiated and differentiated carrot root-callus tissue does not show any significant differences<sup>3</sup>. On the other hand, there are reports that differentiation of organs in the cultured tissue has some influence on its biosynthetic potentiality<sup>4-6</sup>. We have found both qualitative and quantitative differences in the free amino acids of undifferentiated and differentiated stem-callus tissue of *Citrus grandis* (L.) Osbeck, which are reported here.

### EXPERIMENTAL PROCEDURE

Tissue explants were taken from 2 to 2½-year-old stem-callus type-A tissue of *C. grandis*, which had been maintained in modifications of Murashige and Skoog's medium<sup>7</sup>.

For organogenesis, the tissue explants were cultured in another variant of the basal medium<sup>7</sup> with supplements of 0.25 mg/l 6-benzylaminopurine (BAP) + 0.1 mg/l  $\alpha$ -naphthaleneacetic acid (NAA).

Sterilization procedure and other cultural conditions were as reported earlier<sup>8</sup>. Quantitative estimation of free amino acids of callus tissue, at the time of inoculation (undifferentiated tissue) and after its 60-70 days' incubation in the medium supplemented with 0.25 mg/l BAP + 0.1 mg/l NAA (differentiated tissue), was made by paper chromatography adopting the following procedure: known amounts of fresh tissues were extracted in 100 ml 80% ethyl alcohol at room temperature for a week. The extracts were concentrated under reduced

pressure to about ¼th of the initial amount. Each sample was spotted separately on Whatman No. 1 chromatographic paper, which was run first with *n*-butanol : acetic acid : water : : 40 : 10 : 50 in one dimension, and phenol : acetic acid : water : : 74 : 1 : 19.2 in the second dimension. The chromatogram was sprayed with 0.4% ninhydrin in acetone and allowed to develop for 30 min at 60° C. The ninhydrin positive spots were cut and extracted separately in 5 ml 75% ethyl alcohol saturated with CuSO<sub>4</sub>. The extracted colour, after 30 min, was read in Spectronic-20 colorimeter at 540 m $\mu$ , and compared with the standard samples of amino acids subjected to the same treatment.

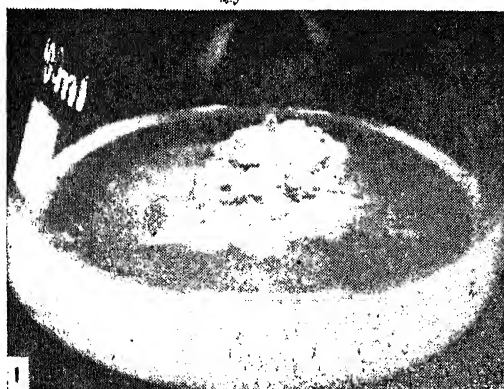
### RESULTS AND DISCUSSION

In the medium supplemented with 0.25 mg/l BAP + 0.1 mg/l NAA, numerous tiny green shoot-buds were formed on the surface of stem-callus tissue of *C. grandis* after an incubation of about 60 days (Fig. 2). Young shoot-buds showed numerous epidermal hairs. Stem-callus tissue, type-A, from which the explants were taken was compact-nodular, greenish-white, and devoid of any shoot-bud (Fig. 1).

Free amino acid analysis of the above-mentioned undifferentiated and differentiated tissue brought out not only quantitative differences, but also qualitative (cf. 3, Table I). Except glycine—the concentration of which remained unchanged, the amounts of all other amino acids increased in the differentiated tissue. This was strikingly high in respect of L-tyrosine which showed 5-fold increase, and L-alanine and L-threonine which showed an increase of 4-fold each. The amounts of L-proline and L-serine got tripled, whereas those of L-aspartic acid, L-glutamic acid and L-tryptophane got doubled (approx.). L-cystine and L-methionine, which could not be traced in the undifferentiated tissue, were present in good amount in the differentiated tissue. On the other hand, L-arginine and L-asparagine, which were present in the undifferentiated tissue, disappeared from the differentiated tissue.

The increase in amounts of amino acids in the differentiated tissue, as compared to the undifferentiated tissue of *C. grandis*, is indicative of higher metabolic activity in the former. There are some instances where differentiating organs in (*in vitro*) growing callus tissue do affect the concentration of plant constituents in them. Stem and leaf-callus tissue of *Atropa belladonna* does not synthesise atropine unless macroscopic roots are formed<sup>5</sup>. On the contrary, diosgenine content of root-callus tissue of *Dioscorea deltoidea* gets reduced following rhizogenesis<sup>6</sup>. In tobacco callus cultures, the high

scopoletin content is correlated with a high capacity to form shoot-buds<sup>4</sup>. In the present study, changes in the concentration of amino acids, as also the appearance of some new amino acids and the loss of some others, are correlated with the differentiation of shoot-buds in the callus tissue. However, it cannot be concluded as to whether such quantitative and qualitative changes in the free amino acid content of the tissue cultured on the medium supplemented with BAP + NAA precede shoot-bud differentiation, or result therefrom.



FIGS. 1-2. Cultures of stem-callus tissue of *Citrus grandis*. Fig. 1. Tissue in undifferentiated state ( $\times 1.2$ ). Fig. 2. Tissue showing differentiation of shoot-buds in the medium supplemented with 0.25 mg/l BAP + 0.1 mg/l NAA ( $\times 9.9$ ).

TABLE I

Free amino acid content of stem-callus tissue of *Citrus grandis*

Amino acid*	Undifferentiated tissue	Differentiated tissue
L-Alanine	16.6	72.0
L-Arginine	18.7	..
L-Asparagine	45.8	..
L-Aspartic acid	32.0	72.0
Glycine	38.2	44.0
L-Glutamic acid	42.0	106.4
L-Proline	16.0	54.0
L-Serine	20.5	62.0
L-Threonine	25.0	100.0
L-Tryptophane	31.6	60.0
L-Tyrosine	12.2	60.0
L-Cystine	..	78.0
L-Methionine	..	40.8

\* Quantity in  $\mu\text{g}/100$  mg fr. wt. of callus tissue; data based on three chromatographs of each sample.

#### ACKNOWLEDGEMENT

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## EFFECT OF DESICCATION TREATMENT ON CATALASE ACTIVITY OF *SESAMUM INDICUM* L. DURING GERMINATION

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### ABSTRACT

Seedlings of *Sesamum indicum* L. were subjected to desiccation treatment. Catalase activity progressively increased with advance in seedling growth. During desiccation period the enzymic activity decreased while after revival it increased considerably.

### INTRODUCTION

**C**ATALASE activity increases during water stress. Oxidation reduction processes occur at faster rates in plants under water stress which is apparently due to an increase in the metabolism but to an unproductive utilization of energy in vital processes<sup>1,2</sup>.

The effect of different periods of desiccation on the enzymic activity of sesamum seedlings is reported here.

### MATERIAL AND METHODS

Graded seeds of *Sesamum indicum* L. var. Kundla were germinated in Petridishes lined with sterilized filter-paper, and the following growth stages of the seedlings were noted.

Stage	Character
I	Root just emerging
II	Root hairs appear in bunch
III	Hypocotyl just protruding
IV	Hypocotyl developing greenish tinge
V	Testa breaking and cotyledons coming out.

The seeds were germinated upto these five growth stages and subjected to two desiccation treatments of 2 and 4 days respectively.

Method of desiccation treatment has been described elsewhere<sup>3</sup>.

Catalase activity was studied in seedlings upto the fifth growth stage. The estimations were carried out: (i) before the initiation of the desiccation treatment, i.e., in the undessicated material, (ii) at the end of the desiccation period, and (iii) after revival, i.e., when the desiccated seedling had reached the next stage of germination to the one at which it was subjected to desiccated treatment. The data were subjected to analysis of variance using Fisher's method (1954)<sup>4</sup>.

Catalase activity was determined by the method of Chance and Maehly (1955)<sup>5</sup> and expressed as  $O_2$  evolved/min/g. dry wt.

### RESULTS

Considering the complex nature of the experiment the results are grouped to bring out the effects of single factors and also of their interactions. Thus, for instance, to obtain the mean values for different germination stages (I  $\rightarrow$  V), all the determinations for the five desiccation treatments and for the three replicates were added up for each germination stage and divided by the total number of determinations, i.e.,  $(5 \times 3) = 15$  for each germination stage. Therefore, in Fig. 1, each of the five histograms for

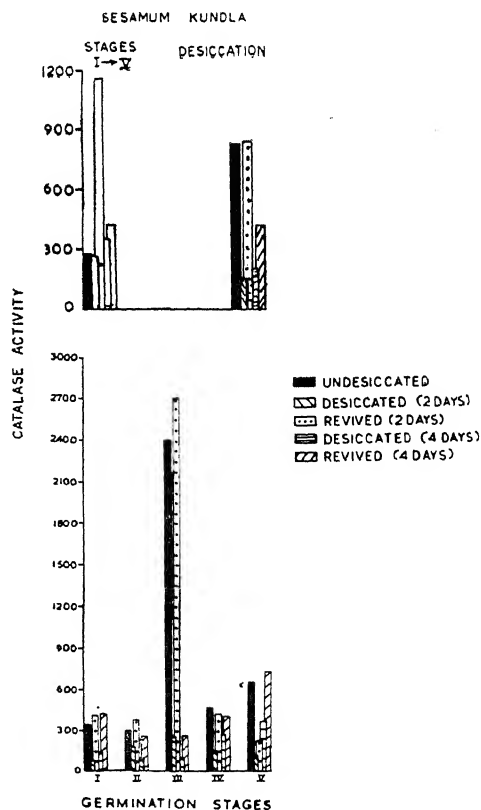


Fig. 1. Catalase activity in *Sesamum indicum* L. var. kundla.

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germination stages is a mean of 15 determinations of catalase activity. Similarly, for the five desiccation treatments, values for the germination stages and for the three replicates were added up for each desiccation treatment and divided by the total number of determinations, i.e.,  $(5 \times 3) = 15$  which gave the mean values for each of the five desiccation treatments.

The following main points emerge from the data :

- (a) There is a progressive increase in the catalase activity with the advance in seedling growth, reaching the maximum value in the third stage.
- (b) During the two desiccation treatments there is a sharp fall in catalase activity which is considerably enhanced in the revived seedlings.
- (c) Seedlings revived after 2-day desiccation treatment register higher catalase activity than the undesiccated seedlings upto the third stage of germination. In both cases the highest value is reached in the third stage. In the later stages, 4-day desiccation treatment and its revival appear more beneficial for catalase activity as compared to 2-day desiccation treatment and its revival.

Analysis of variance of the data (Table I) shows that effects of desiccation treatments are highly significant.

TABLE I

Analysis of variance of data of catalase activity in *Sesamum indicum*, var. kundla

Factor	Degree of freedom	Variance	F value
Stages (St)	4	544321.9	2.0
Desiccation treatment (Dt)	4	2200879.6	8.5*
Replicates	2	78.6	0.0
St $\times$ Dt	16	283312.9	1.1
Error	49	256966.9	..
Total	75	..	..

\* Denotes significant effects of treatments at 1% P.

#### DISCUSSION

Increased catalase activity during germination indicates that the catabolic processes are dominant and the resulting breakdown products

function as precursors for the biosynthesis of different metabolites and thus fulfill the energy requirement of the growing seedling.

Increased catalase activity indicates an enhancement in the oxidative activities which is suggestive of the shift in the redox balance of the system to the oxidative side. Thus the increase in the general metabolism of the desiccated seedling caused by the oxidative shift of the redox balance is at the expense of the breakdown products of essential metabolites and the energy liberation being unproductive<sup>2</sup>. As the catabolic processes surpass the anabolic processes, the growth of the seedling becomes standstill. This is evident from the fact that during desiccation period no further growth of the seedling is observed, i.e., during 2 or 4 days of desiccation the seedling remains at the same stage at which it was desiccated.

Jaikaria (1971)<sup>6</sup> observed that in *Cicer* seedlings, the enhanced catalase activity parallels with the greater utilization of ascorbic acid during desiccation, suggesting that the faster production of  $H_2O_2$  during faster ascorbic acid utilization is used as a substrate by increased catalase and peroxidase activities, thereby affording protection to the plant against peroxidative damages.

These observations indicate that desiccation treatment of suitable period increases the metabolism of the germinating seedlings, thus helping the plant to successfully tide over the unfavourable conditions of water stress.

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## LETTERS TO THE EDITOR

FLUOROMETRIC STUDY OF 5 METHOXY-3  
PHENYL-4 METHYL COUMARIN

THE present study relates to the effect of solvent on the spectral behaviour of 5 methoxy-3 phenyl-4 methyl coumarin. The compound was prepared and purified by chromatography<sup>1</sup>. The fluorescence and absorption spectra of this compound were recorded at room temperature with Aminco-Bowman Spectrophotofluorometer and Perkin-Elmer 4000 Å spectracord respectively. The concentration of the fluorescent solute in solution was kept low ( $\sim 5 \times 10^{-6}$  g/cc) to minimise the effect of self-quenching. The fluorescent maximum ( $\bar{\nu}_f$ ), absorption maximum ( $\bar{\nu}_a$ ) and the relative quantum yield  $Q_r$  are quoted in Table I.

TABLE I

Solvent	Absorption Maximum ( $\bar{\nu}_a$ )	Fluorescent Maximum ( $\bar{\nu}_f$ )	$Q_r$
Water ..	32788 cm <sup>-1</sup>	22649 cm <sup>-1</sup>	0.6
Ethanol ..	33057	25195	0.4
Methanol ..	33222	25221	0.3
Chloroform	33002	25415	0.2
Dioxane ..	33334	25515	0.15

It can be seen from the table that for the change of solvent from a proton donating like water to a proton accepting like chloroform there is a variation of less than 1% in  $\bar{\nu}_a$  whereas  $\bar{\nu}_f$  varies by more than 11% and  $Q_r$  by 300%. The effect of solvent on solute molecules is electrostatic in nature and is essentially due to a dipole-dipole interaction or due to hydrogen bonding and intramolecular charge transfer. If the solute molecules become more polar in the excited state, a greater electrostatic stabilization of the excited state relative to the ground state takes place. Hence, a red shift in  $\bar{\nu}_a$  is observed with increased polarity of the solvent. In such a case, a thermal relaxation of the Frank-Condon excited state further lowers the electronic energy of the state and shifts  $\bar{\nu}_f$  to a longer wavelength. These changes in  $\bar{\nu}_a$  and  $\bar{\nu}_f$  are further enhanced by hydrogen bonding. Hydrogen bonding causes greater electrostatic stabilization of the excited state. This is so because the hydrogen bond donor solvents increase charge transfer by introducing a partial positive charge into the charge transfer acceptor groups. In view of the above discussion, the observed dependence of  $\bar{\nu}_a$  and  $\bar{\nu}_f$  (Table I) on the nature of the solvent—proton donating (*viz.*, water, ethanol, methanol) or proton

accepting (*viz.*, chloroform, dioxane)—may be attributed to the formation of hydrogen bonds.

It is also clear from the table that the value of  $Q_r$  changes with the solvent. The value of  $Q_r$  depends on the positions of  $n\pi^*$  and  $\pi\pi^*$  singlets and triplets. Hydrogen bonding affects the relative positions of these levels. In coumarins, as in other fluorescent molecules,  $\pi\pi^*$  is the lowest singlet. Hydrogen bonding perturbs the close lying  $\pi\pi^*$  and  $n\pi^*$  levels of the coumarin molecule. If this increases the energy gap between  $S\pi\pi^*$  and  $Tn\pi^*$ , and lowers  $S\pi\pi^*$  still leaving it as the lowest singlet, the quantum yield of fluorescence would be enhanced<sup>2,3</sup>. At the same time  $\bar{\nu}_f$  which is a measure of the energy  $\pi \leftarrow \pi^*$  transition will be lowered. It appears that this is what is happening in the present case, the observed values of  $Q_r$  being higher and  $\bar{\nu}_f$  lower in case of hydroxyl group carrying solvents (capable of forming hydrogen bonds) than in non-hydroxyl group carrying solvents (incapable of forming hydrogen bonds). It appears therefore that the effect of solvent on  $n\pi^*$  level is not large enough to bring it below  $\pi\pi^*$  level.

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Astrophysics,  
University of Delhi,  
Delhi, March 2, 1974.

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J. KISHORE,  
M. K. MACHWE,  
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ROTATIONAL ANALYSIS OF THE (0, 0) BANDS  
OF THE  $C^2\Pi \rightarrow X^2\Sigma^+$  SYSTEM OF CaBr  
MOLECULE

THE spectrum of CaBr molecule has been studied by various workers<sup>1-4, 7-9</sup> and the vibrational analysis of  $A \rightarrow X$ ,  $B \rightarrow X$ ,  $C \rightarrow X$ ,  $D \rightarrow X$  and  $E \rightarrow X$  has been carried out. The rotational analysis of none of these systems has been carried out so far. The present note deals with the rotational analysis of the  $C \rightarrow X$  system of CaBr molecule. The molecular constants derived from the present work are also reported.

The spectrum of CaBr was excited in a high frequency discharge. External heating was found necessary to maintain the characteristic red colour of the discharge. The (0,0) bands of the C-system of this molecule (3910–3960 Å) were photographed in the sixth order of a 2-metre plane grating

spectrograph at a reciprocal dispersion of 0.78 Å/mm.

The author is grateful to Dr. M. M. Patel for suggesting the problem and useful discussions.

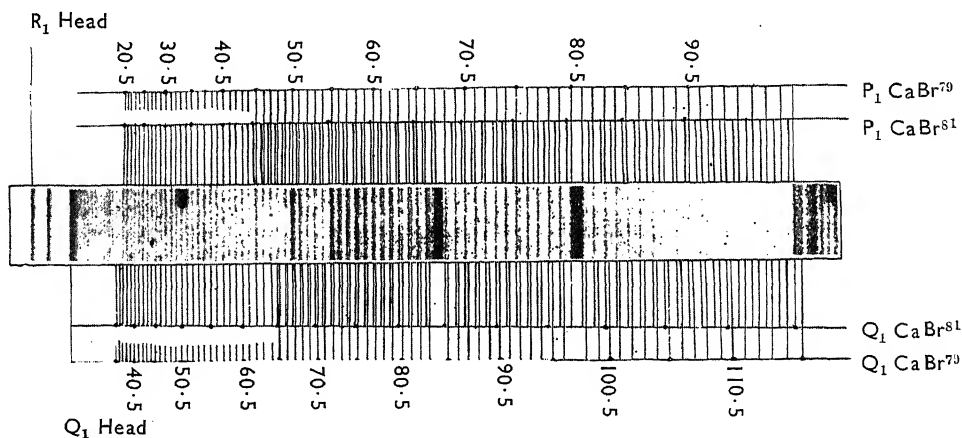


FIG. 1. (0,0) Band of the  $C^2\Pi \rightarrow X^2\Sigma^+$  system of CaBr molecule.

The (0,0) band at 3950 Å (Fig. 1) has been clearly resolved showing the presence of  $P_1$ ,  $R_1$  and  $Q_1$  branches of which  $Q_1$  and  $R_1$  form the respective band heads. The rotational isotopic shift due to bromine has also been exhibited by  $P_1$  and  $Q_1$  branches. The spectrogram of the band at 3916 Å clearly reveals the presence of well-resolved  $P_2$  and  $Q_2$  branches in addition to the unresolved  $R_2$  branch and a satellite branch  $R_{21}$ , out of which  $R_2$  and  $R_{21}$  form the respective band heads. The  $P_2$  and  $Q_2$  branches also show the rotational isotopic shift due to bromine.

The fine structure analysis of the above mentioned bands was carried out by following the standard methods (Herzberg, 1950). The correct J assignments to the rotational lines were made by comparing the combination differences for the common lower states involved in both the bands assuming that the  $\Lambda$ -type doubling in the upper state is too small to be accounted for. The criterion suggested by Youngner and Winans (1960) was also applied to check the correctness of the J assignments.

From the analysis it has been confirmed that the two bands at 3950 Å and 3916 Å are the (0,0) bands of the C-system of CaBr molecule due to an electronic transition,  $C^2\Pi \rightarrow X^2\Sigma^+$ . The rotational constants derived in the present investigation are given below:

CaBr <sup>81</sup>	CaBr <sup>79</sup>
$B_0' = 0.0764 \pm 0.0005 \text{ cm}^{-1}$	$B_0' = 0.0758 \pm 0.0005 \text{ cm}^{-1}$
$D_0' = 2.4 \times 10^{-8} \text{ cm}^{-1}$	$D_0' = 2.4 \times 10^{-8} \text{ cm}^{-1}$
$r_0' = 2.88 \text{ Å}$	$r_0' = 2.88 \text{ Å}$
$B_0'' = 0.0816 \pm 0.0005 \text{ cm}^{-1}$	$B_0'' = 0.0811 \pm 0.0005 \text{ cm}^{-1}$
$D_0'' = 2.4 \times 10^{-8} \text{ cm}^{-1}$	$D_0'' = 2.4 \times 10^{-8} \text{ cm}^{-1}$
$r_0'' = 2.78 \text{ Å}$	$r_0'' = 2.78 \text{ Å}$

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## STUDIES ON FLUOROBERYLLIC ACID AND ITS SALTS

ANALOGY of fluorobaryllates with sulphates has been shown by Ray<sup>1</sup>. Free fluoroberyllic acid was isolated by ion exchange method<sup>2</sup>. In the present investigation the dissociation constant of  $H_2(BeF_4)$  and ionic mobility of fluoroberyllate ion have been evaluated. Solubilities of sparingly soluble salts of the acid have also been determined.

### First Dissociation Constant of Fluoroberyllic Acid

1. *Potentiometry*.—Potentiometric titrations were carried out using a Cambridge pH-meter fitted with a quinhydrone electrode. The solution of fluoroberyllic acid in a polythene beaker was titrated with standard sodium hydroxide. Eight such titrations were carried out.

Boral, Saha and Ray<sup>3</sup> have shown that  $BeF_3$  in aqueous solution forms stable auto-complex

[Be(H<sub>2</sub>O)<sub>4</sub>]. Hence further dissociation of BeF<sub>4</sub> into BeF<sub>2</sub>+2F<sup>-</sup> and of BeF<sub>2</sub> into Be<sup>++</sup> and 2F<sup>-</sup> in a solution of fluoroberyllic acid is not possible.

The average value for K<sub>1</sub> was found to be  $3.46 \times 10^{-3}$  and the mean deviation being  $0.7 \times 10^{-3}$ . The large variation in the values from the average may be attributed to the overlapping dissociation<sup>5</sup> which takes place when the first and second dissociation constants do not differ by more than 10<sup>3</sup>.

2. *Cryoscopy*.—Cryoscopic experiment was carried out with water as the solvent. The acid did not affect glass at high dilution.

The following results were obtained :

Room temperature .. 26° C,  
Strength of fluoroberyllic acid .. 2.55%

Mean  $\Delta T_f$  for 2.55% fluoroberyllic acid = 1.61°

Apparent Mol wt. = 29.47

Degree of dissociation = 0.9765

Dissociation constant =  $2.37 \times 10^{-3}$

The average value of three determinations of the first dissociation constant of fluoroberyllic acid was in fair agreement with that obtained by the potentiometric method.

#### Ionic Mobility of Fluoroberyllate Ion

The ionic mobility of fluoroberyllate ion was obtained from the conductance of the sodium, potassium and ammonium salts of the acid.

The equivalent conductances of sodium and potassium salts of the acid were determined with paraffin coated conductivity cell at different concentrations and extrapolated to infinite dilution.

Substance used	Equiv. cond. at infinite dilution Ohm <sup>-1</sup>	Ionic cond. of cations <sup>5</sup> Ohm <sup>-1</sup>	Ionic cond. of BeF <sup>-2</sup> Ohm <sup>-1</sup>	Ionic mobility of BeF <sub>4</sub> <sup>-2</sup>
Sodium fluoro-beryllate	112	43.3	68.60	$7.1 \times 10^{-4}$
Potassium fluoro-beryllate	133.2	64.6	68.60	$7.1 \times 10^{-4}$
Ammonium fluoroberyllate	134.1	64.3	68.60	$7.2 \times 10^{-4}$

#### Solubilities of Difficultly Soluble Salts of Fluoroberyllic Acid

The specific conductances of the saturated solutions were calculated, after deducting the value for pure water.

#### Results

Solubilities of fluoroberyllates of	S $\times 10^{-3}$
Calcium	.. .. 1.67
Barium	.. .. 1.25
Strontium	.. .. 1.43
Lead	.. .. 1.88

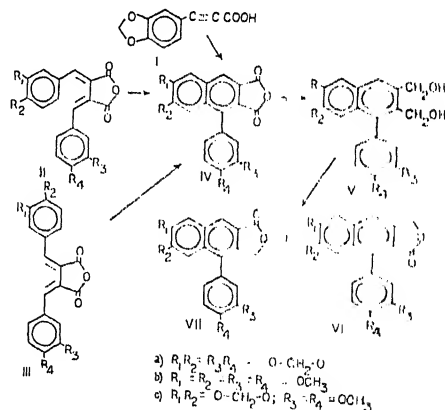
Author's grateful thanks are due to Dr. N. N. Ray formerly Reader in Chemistry, University College of Science, Calcutta, for his guidance and interest in the work, and to Dr. T. N. Chakravarty for his help.

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#### A NEW SYNTHESIS OF JUSTICIDIN E AND TAIWANIN C

RECENTLY, Holmes and Stevenson<sup>1</sup> reported the synthesis of justicidin E (VII a)<sup>2</sup> and taiwanin C (VI a)<sup>3</sup> from 1-piperonyl propiolic acid (I). A facile alternate synthesis of these lignins is now reported starting from *trans*, *trans*-dipiperonylidene-succinic anhydride (III a).



The successful use of Pb(OAc)<sub>4</sub>-AcOH to cyclise diarylidenesuccinic anhydrides was recently reported 'from these laboratories'<sup>4</sup>. But, this reagent had no effect on *trans*, *trans*-dipiperonylidene-succinic anhydride (III a) under varying conditions. The *cis*, *trans*-isomer (II a), which accompanied the former (III a) as a minor product in its synthesis<sup>5</sup>, cyclised in 5 hrs and the yield of 1-piperonyl naphthoic anhydride (IV a) was 50%. After several trials, it was observed that addition of a trace of HClO<sub>4</sub> to the reaction mixture caused the ring closure of III a in 1.5 hrs to give IV a in 60%. Under similar conditions, cyclisation of the isomer (II a) was complete in 1 hr and the yield of IV a was 80%.

6, 7-methylenedioxy - 1-(3', 4' - methylenedioxyphenyl) naphthalene-2, 3-dicarboxylic acid anhydride (IV *a*) crystallised as yellow needles, m.p. 222–225° from acetone<sup>5</sup> and m.p. 240–242° from chloroform-methanol<sup>6</sup>. IR (CHCl<sub>3</sub>): 1845 and 1780 cm<sup>-1</sup> (anhydride); Found: C, 66.28; H, 3.0; C<sub>20</sub>H<sub>10</sub>O<sub>7</sub> requires C, 66.31 and H, 2.78%.

The use of HClO<sub>4</sub> resulted in effecting the ring closure of the resistant *trans*, *trans*-isomer, and also improved the yields and reduced the reaction times considerably. It was next extended to the cyclisation of symmetrical diveratrylidenesuccinic anhydrides (II *b* and III *b*) and also to the unsymmetrical

colourless needles, m.p. 270–272° (lit.<sup>1</sup> 271–272°), IR (CHCl<sub>3</sub>): 1755 cm<sup>-1</sup> (lactone) and was identical (mmp & IR) with an authentic sample of justicidin E, kindly gifted by Dr. Stevenson. The isomeric lactone (IV *a*, yield 16%) crystallised from benzene as colourless prisms, m.p. 275–276° (lit.<sup>1</sup> 272–276.5°), IR (CHCl<sub>3</sub>): 1760 cm<sup>-1</sup> (lactone) and agreed in all its physical characteristics recorded in literature<sup>1,3</sup> for taiwanin C.

The two naphthoic anhydrides (IV *b* and IV *c*) were also similarly converted into the corresponding pairs of lactones, through the diols V *b* and V *c* (Table I).

TABLE I

Naphthoic anhydride	Diol	Lactones	
		A	B
IV <i>b</i>	V <i>b</i>	VI <i>b</i>	VII <i>b</i>
m.p. 310–312° (lit. <sup>1</sup> 305–306°)	m.p. 189–190° (lit. <sup>1</sup> 189–190°)	m.p. 253–255° (lit. <sup>1</sup> 253–255°)	m.p. 216–217° (lit. <sup>1</sup> 215–216°)
IV <i>c</i>	V <i>c</i>	VI <i>c</i>	VII <i>c</i>
m.p. 270–271° (lit. <sup>4</sup> 270°)	m.p. 180–182° (lit. <sup>4</sup> 179–180°)	m.p. 229–230°	m.p. 240–242°

veratrylidene, piperonylidenesuccinic anhydride (II *c*). In the former case, the mixture of II *b* and III *b* from Stobbe condensation was not separated, but warmed with Pb(OAc)<sub>4</sub>–HOAc and a trace of HClO<sub>4</sub>. The cyclisation was complete in 15 mts and the yield of IV *b*<sup>4</sup> was 90%.

In the case of the unsymmetrical veratrylidene, piperonylidenesuccinic anhydride, only the *cis*, *trans*-isomer (III *c*)<sup>4</sup> was formed in the Stobbe condensation and it cyclised in 1.5 hrs to give 80% of the naphthoic anhydride IV *c*<sup>4</sup>. It may be pointed out here that the course of cyclisation of III *c* was not altered by the use of HClO<sub>4</sub> and the naphthoic anhydride was identical with that obtained earlier<sup>4</sup>.

The conversion of IV *a* to justicidin E and taiwanin C was next effected following the method adopted by Holmes and Stevenson<sup>1</sup>. Reduction of the naphthoic anhydride (IV *a*) to the diol (V *a*) was accomplished by LAH in THF at reflux temperature and the diol (V *a*) crystallised from benzene, m.p. 188–189°, IR (CHCl<sub>3</sub>): 3400 cm<sup>-1</sup>. It was then refluxed with silica gel-Ag<sub>2</sub>CO<sub>3</sub> instead of celite-Ag<sub>2</sub>CO<sub>3</sub> (cf. Ref. 1) in benzene solution for 2 hrs and the resulting isomeric lactones (VI *a* and VII *a*) were separated by column chromatography over silica gel using benzene as eluent. The lactone (VII *a*, yield 30%) crystallised from benzene as

The structure of the lactones (VI *c* and VII *c*) were assigned from a study of their <sup>1</sup>H NMR spectra in which significant difference in the chemical shifts can be expected for the lactone methylene protons. The compound with m.p. 229–230° (IR, 1750 cm<sup>-1</sup> for the lactone; Found: C, 69.60; H, 4.38; C<sub>21</sub>H<sub>16</sub>O<sub>6</sub> requires C, 69.23 and H, 4.43%) is assigned the structure VI *c*, for the lactone methylene protons appeared as a singlet at δ 5.31 as in taiwanin C (IV *a*)<sup>1</sup>. In the isomeric lactone, m.p. 240–242°, (IR, 1755 cm<sup>-1</sup> for lactone, Found: C, 69.03; H, 4.61; C<sub>21</sub>H<sub>16</sub>O<sub>6</sub> requires C, 69.23 and H, 4.43%), the lactone methylene protons appeared high field at δ 5.13 s as in justicidin E (VII *a*)<sup>1</sup> and therefore it is represented by the alternate structure VII *c*.

Two of us (V. K. R. and A. M. R.) wish to express our grateful thanks to CSIR, New Delhi, for the Fellowships. Our thanks are also due to Professor N. V. Subba Rao, Osmania University, Hyderabad, for the NMR spectra.

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February 27, 1974. L. RAMACHANDRA ROW.

Note added in proof:

We came across a recent publication (B. J. Arnold, S. M. Mellows and P. G. Sammes, *J. Chem.*

Soc., Perkins I, 1973, p. 1266) in which a new synthesis of the title compounds was reported by a longer and arduous route (about 12 stages).

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### ANTHOCYANIN OF SUGARCANE

In an earlier note<sup>1</sup> it was mentioned that the peelings of sugarcane contain two flavone glycosides. We now report the isolation of an anthocyanin from this plant.

The reddish brown peelings of sugarcane were extracted with ethanolic hydrochloric acid at room temperature. The concentrate was kept in the cold and the residue thus obtained crystallised from alcohol-HCl mixture as chocolate coloured crystals. It gave positive Molisch test and other reactions characteristic of anthocyanins.

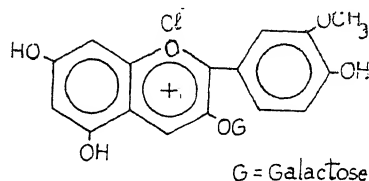
The glycoside on hydrolysis with 20% HCl yielded an anthocyanidin, which could be precipitated with petroleum ether from amyl alcohol solution into 1% aqueous HCl layer. It crystallised as dark reddish brown prisms from ethanolic-hydrochloric acid mixture.

From the hydrolysate, a sugar was isolated which was identified as D (–) galactose by its osazone and co-chromatography with an authentic sample. Quantitative sugar estimation showed it to be a mono-galactoside.

Various colour tests indicated that the anthocyanin or its aglycone did not contain free vicinal hydroxyl groups in the attached benzene ring. Quantitative estimation by Ziesel method confirmed the presence of one methoxyl group. The anthocyanidin and anthocyanin both gave specific colour reactions of peonidin derivatives and had  $\lambda_{\max}$  at 542 and 532 m $\mu$  respectively in ethanolic solution. A hypsochromic shift of 10 m $\mu$  in the glycoside<sup>2</sup> was an indication of the probable position of sugar at 3. The peonidin structure was further supported by no shift in the  $\lambda_{\max}$  for either glycoside or aglycone with 1%  $\text{AlCl}_3$  reagent.

Oxidation of anthocyanidin with  $\text{KMnO}_4$  yielded vanillic acid and its alkali fission gave phloroglucinol,

both identified by co-chromatography on paper and mixed m.p. with authentic samples. Finally the anthocyanidin was confirmed as peonidin by co-chromatography on paper using Forrestal solvent with an authentic sample of peonidin isolated from fresh viola tricolor flowers. Hence the structure (I) has been assigned to the anthocyanin from sugarcane.



(I)

It is for the first time that peonidin-3-mono-galactoside is being reported in this plant, although the presence of delphinidin, indicated only by colour tests, has been reported earlier in this source<sup>3</sup>.

We convey our thanks to Prof. R. D. Tewari for his interest in the present work and also to SCSIR (U.P.) for the grant of assistantship to R. C. D.

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Allahabad, June 18, 1974.

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### ISOLATION OF 3, 3', 4-TRI-O-METHYL-FLAVELLAGIC ACID FROM BARK OF *ANOGEISSUS LATIFOLIA*

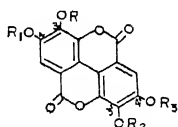
*Anogeissus latifolia* is a large and moderate sized tanniferous tree<sup>1</sup> and earlier workers<sup>2</sup> isolated ellagic acid (I) and 3, 3', 4-tri-O-methyl-ellagic acid (II) from the bark. By continuous soxhlet extraction of the powdered bark with chloroform, it has now been possible to isolate a yellow compound, m.p. 280–1° (dioxane);  $M^+$  360,  $\text{C}_{17}\text{H}_{12}\text{O}_9$ ;  $\nu_{\max}^{\text{nujol}}$  1685 ( $>\text{C}=\text{O}$ ), 1705 ( $>\text{C}=\text{O}$ ), 3280 ( $-\text{OH}$ )  $\text{cm}^{-1}$ ;  $\lambda_{\max}^{\text{EtOH}}$ , 245 (4.60), 366 (4.00), 382 (4.04) nm. These absorptions are highly charac-

teristic of ellagic acid (I) and flavellagic acid (IV) derivatives<sup>3,4</sup>.

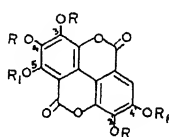
Further,  $\text{AlCl}_3$  brings about the shift of longer wavelength band (382) by 35 nm which reveals the presence of a chelated hydroxyl-carbonyl system. The absence of shift with boric acid indicates the absence of vicinal hydroxyl groups in the molecule. Since the lower wavelength band (245) has not undergone a shift with sodium acetate and since no prominent red colour is observed in Griessmayer test<sup>5</sup>, at least one or both of 4, 4'-hydroxyl groups of ellagic (I) or flavellagic (IV) acid type may be as methyl ether.

On treatment of the compound with acetic anhydride and pyridine at room temperature for 48 hours, it has yielded a diacetate (V), m.p. 242–4°;  $\text{C}_{21}\text{H}_{16}\text{O}_{11}$ ;  $\nu_{\text{max}}^{\text{nuol}}$  1610, 1757  $\text{cm}^{-1}$ ;  $\lambda_{\text{max}}^{\text{EtOH}}$  245 (4.79), 342 (4.13), 355 (4.17) nm; nmr data (60 MHz, TMS,  $\text{C}_7\text{D}_5\text{N}$ ) of the acetate:  $\delta$  8.18 (S, 1 H, aromatic H),  $\delta$  4.27 (S, 3 H,  $\text{OCH}_3$ ),  $\delta$  4.25 (S, 3 H,  $\text{OCH}_3$ ),  $\delta$  4.22 (S, 3 H,  $\text{OCH}_3$ ),  $\delta$  2.58 (S, 3 H,  $\text{OCOCH}_3$ ),  $\delta$  2.43 (S, 3 H,  $\text{OCOCH}_3$ ). All the above data suggest that the yellow compound may be flavellagic acid tri-O-methyl ether. The compound underwent methylation with dimethyl sulphate, potassium carbonate in acetone to give rise to a dimethyl ether (VI), m.p. 252–3°,  $\text{C}_{19}\text{H}_{14}\text{O}_9$ .

Though flavellagic acid and some of its derivatives were prepared synthetically<sup>6</sup>, they have been isolated from natural sources only in 1965. Ramachandra Row *et al.*<sup>7</sup> isolated flavellagic acid derivative from *Terminalia paniculata* heartwood, which was assigned the structure 3,3',4-tri-O-methyl-flavellagic acid (VII). The identity of our compound with it was established by superimposable ir spectrum obtained through the courtesy of Prof. L. Ramachandra Row. The dimethyl ether is found to be identical with penta-O-methyl flavellagic acid (VI), and differed from III.



- I.  $R = R_1 = R_2 = R_3 = \text{H}$   
II.  $R = R_1 = R_2 = \text{CH}_3$ ,  $R_3 = \text{H}$   
III.  $R = R_1 = R_2 = R_3 = \text{CH}_3$



- IV.  $R = R_1 = \text{H}$   
V.  $R = \text{CH}_3$ ,  $R_1 = \text{COCH}_3$   
VI.  $R = R_1 = \text{CH}_3$   
VII.  $R = \text{CH}_3$ ,  $R_1 = \text{H}$

In the mass spectrum of the compound, wherein the molecular ion (360) is itself the base peak, the other prominent fragments obtained due to the loss of carbon monoxide and methyl radicals are  $m/e$  345 (M–15), 317 (345–28), 330 (345–15),

315 (330–35), 299 (330–31), 287 (315–28). The mass spectral fragmentation also supports the structure (VII) assigned to the compound.

The authors are grateful to Prof. M. R. Suxena for his generous assistance in procuring and identifying the plant material, to Prof. L. Ramachandra Row, Andhra University, Waltair, for the samples of ellagic acid and flavellagic acid methyl ethers and to Prof. N. S. Wulfson, Moscow, for the mass spectrum.

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## NITRATE REDUCTASE IN RELATION TO NODULATION IN COWPEAS AND MUNG BEANS

GRAIN legumes including chick peas (*Cicer arietinum*), red gram (*Cajanus cajan*), cowpeas (*Vigna sinensis*), mung beans (*Phaseolus radiatus*) and others largely depend upon their own nitrogen fixation ability to meet their nitrogen requirements<sup>1</sup>. There is usually a lag of 15 to 30 days between germination and the establishment of nitrogen fixation system in most grain legumes. The growth rates during this period are slow and possibly the plant must depend on the soil nitrogen during this period. This would necessitate the functioning of nitrate reductase in these plants. How nitrate reductase activity functions in relation to nodulation is not known. We here report the nitrate reductase activity during growth and development of cowpeas and mung beans in relation to nodulation.

Cowpeas cv C 152 and mung beans cv Hybrid 45 were grown under field conditions from July to early October, 1973. Leaf samples for nitrate reductase assay from the topmost fully expanded leaves were taken at weekly intervals from the time of germination to fruit development. Nitrate reductase was assayed by the *in vivo* method of Klepper *et al.*<sup>2</sup>. Weekly samples of 15 plants in three groups of each were uprooted carefully to determine nodule number and weight,

Both in mung beans and cowpeas, very high nitrate reductase activity was observed in cotyledonary leaves (Fig. 1). The enzyme activity came down to the lowest level by 3rd week but started rising again and reached another peak in the fifth week. There was a rhythmic behaviour in nitrate reductase in both cowpea and mung beans.

9th week, there were very few nodules left on the plants. It was at this time when the third and fourth peaks of nitrate reductase activity in mung beans and cowpeas appeared.

Nitrate reductase is substrate inducible and is considered an important enzyme in relation to protein and grain yield in cereals<sup>3</sup>. High nitrate

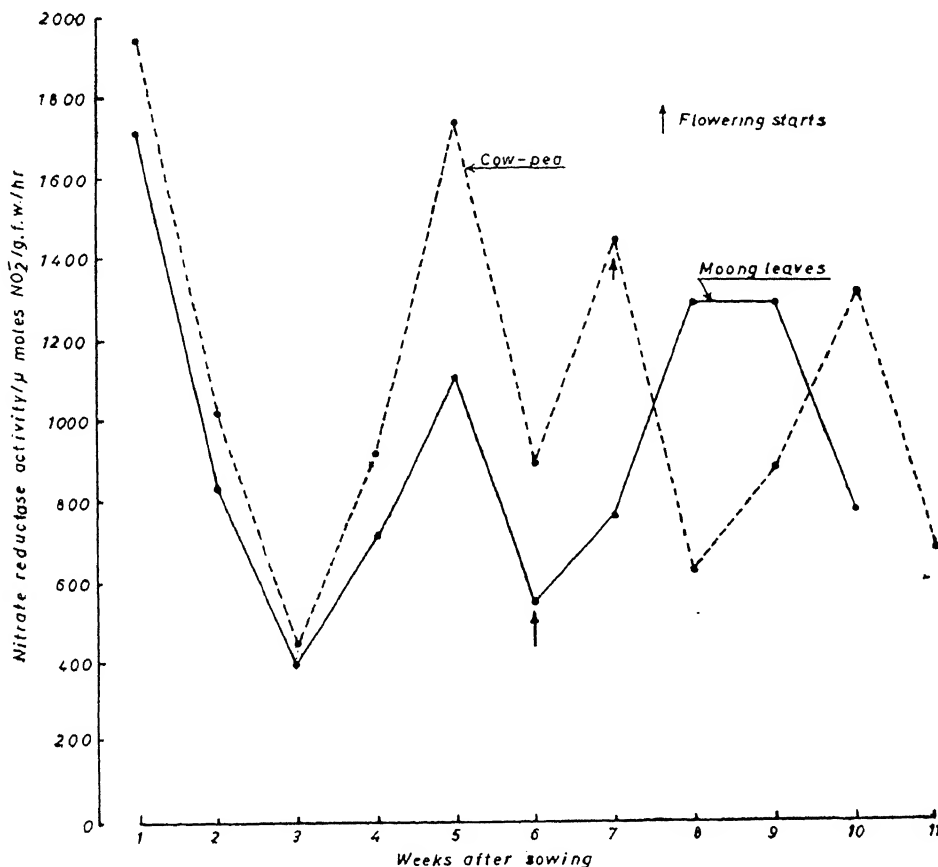


FIG. 1. Nitrate reductase activity during growth of cowpea and mung leaves.

Root nodules appeared in both cowpeas and mung beans by 2nd week after sowing and their number reached the peak in 4th and 5th week respectively (Fig. 2). In cowpeas, the nodule dry weight peak was recorded in 6 weeks while in mung beans the nodule dry weight was maximum in 5 weeks. Thus, it appears that in cowpeas there is a lag between the increase in number and the growth of nodules. However, in mung beans the two processes are probably simultaneous.

It is important to note that both in cowpeas and mung beans by the time flowering started the number and weight of nodules already started declining. In fact, when the flowering was profuse by 8th and

reductase activity prior to the termination of nodulation possibly indicates the source of nitrogen as nitrates from the soil. Is the existence of rhythm in nitrate reductase activity a characteristic of these plants or associated with the formation and decay of nodules? In the latter event the senescent nodules rich in nitrogen may disintegrate to produce nitrate. Therefore, the entire nitrogen fixed by nodules in reduced form may not be used as such but converted to nitrate form before utilization by the plant. This would necessitate re-evaluation of energy requirement of the plant in terms of free nitrogen fixation and utilization as worked out by Bergerson<sup>4</sup>. Furthermore, we will need an evalua-

tion of nitrate reductase activity in leguminous crops besides their nodulation and nitrogen fixation ability,

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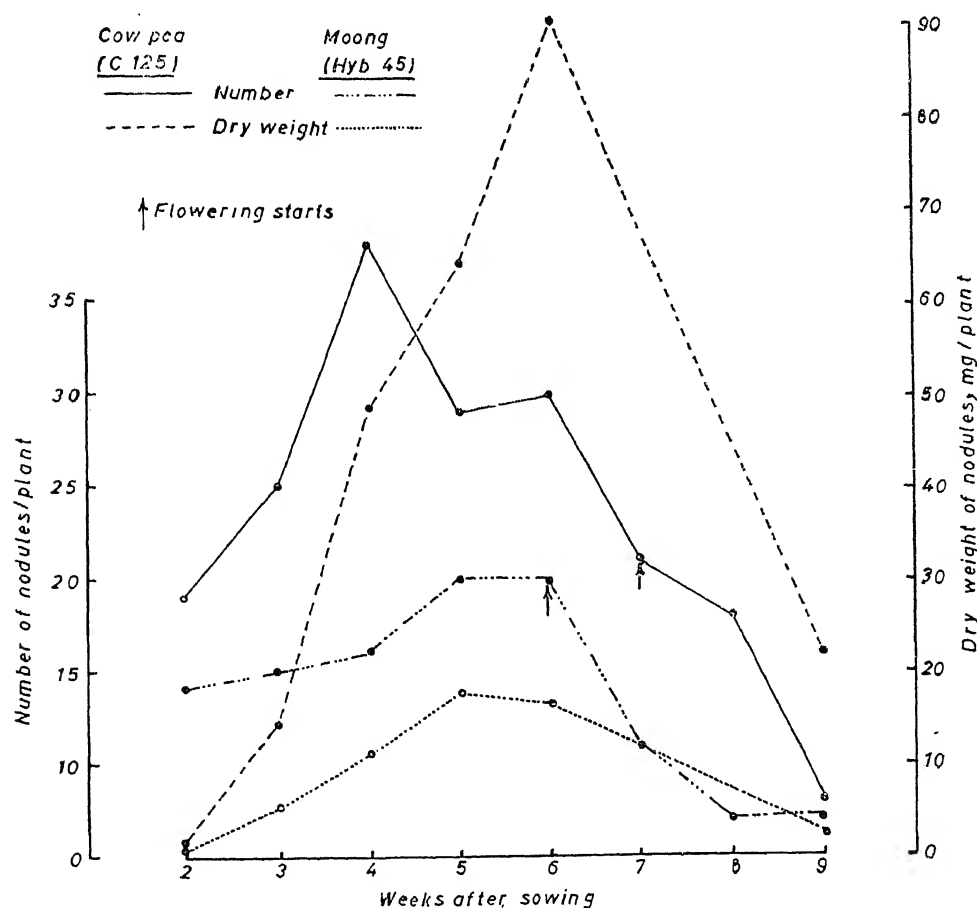


FIG. 2. Nodule number and weight during growth in cowpea and mung.

#### ACKNOWLEDGEMENTS

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#### NODULATION OF *TRIFOLIUM ALEXANDRINUM* BY PENICILLIN TREATED *RHIZOBIUM TRIFOLI*

It has been observed by Hamatova (Personal Communication) that the efficiency of rhizobia increased when grown on a medium containing penicillin. It is also known that additions of alkaloids or high amounts of yeast extract to yeast extract mannitol (YEM) broth induced the formation of bacteroid-like cells in *in vitro* cultures of *Rhizobium*<sup>1</sup>. These findings prompted us to study the influence of penicillin on the morphology of *R. trifolii* and the effectiveness of penicillin treated bacterial cells in nodulating Egyptian clover or

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berseem (*T. alexandrinum*). In one of the treatments, the normal content of yeast extract in YEM medium (0.1%) was raised to 0.35% and in the other treatment penicillin (as benzyl chloride) was added at 500 I.U./ml to study the effects on morphology of cells (light microscopy), growth (turbidimetry) and viability (plate counts) of *R. trifolii*. Unwashed cells and cells washed repeatedly by centrifugation with normal sterile saline water (to get rid of the antibiotic) were used separately to inoculate berseem seedlings grown on Jensen's nitrate-free agar slopes<sup>2</sup> incubated in an illuminated growth room ( $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ; 12 hr photoperiod) under bacteriologically controlled conditions.

The results revealed that penicillin induced the production of bacteroid-like cells reminiscent of the earlier observation with yeast extract<sup>1</sup>. Growth was exponential upto 6 days although less in yeast extract and penicillin added cultures. However, viability of cells was reduced linearly after 24 hr (Fig. 1). Interestingly enough, it was noticed that

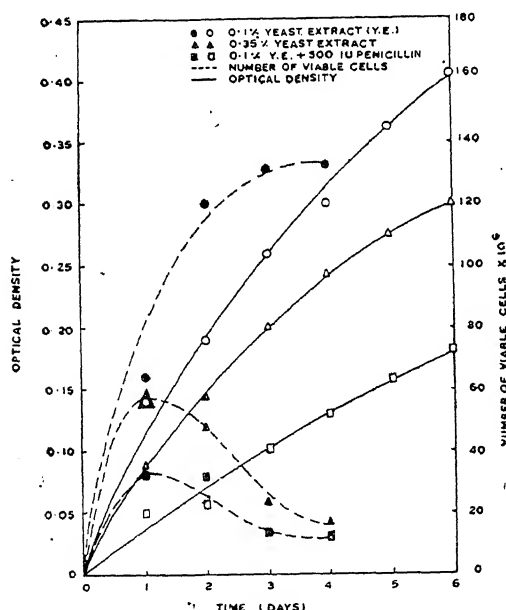


FIG. 1. Growth and viability of *R. trifolii* in the presence of yeast extract and penicillin.

penicillin-treated cells produced more number of nodules resulting in better seedling growth than the untreated bacteria. There was, however, no difference between washed and unwashed cells in their nodulation effects indicating that the residual penicillin had no additional influence (Table I). In our studies, penicillin-treated *Rhizobium* cells (at 500 I.U./ml) grew well after subculturing on penicillin-free medium thereby precluding the

TABLE I

Effect of pretreatment of *R. trifolii* (for 72 hrs) with penicillin on growth and nodulation of *T. alexandrinum* (mean of 12 replicates)  
Data taken at the end of 30 days of plant growth

Penicillin (I.U.)	Nodule number		Plant length (cm)		Plant fresh weight (mg)	
	A	B	A	B	A	B
0	4.2	5.3	24.0	19.8	120.0	101.0
100	5.2	C	26.0	C	139.0	C
300	6.4*	9.8*	23.2	21.7	114.0	112.0
500	7.4*	8.8*	25.8	28.0*	154.0*	154.0*
* C.D. at 5%	2.0	3.5	4.0	4.4	30.0	42.0

A—in seedlings inoculated with washed bacterial cells; B—in seedlings inoculated with unwashed bacterial cells; C—not tried.

mutagenic effects of the antibiotic on the bacterium. On the other hand, penicillin is known to inhibit specific steps involved in cell wall synthesis in bacteria<sup>3</sup>. Certain physico-chemical changes in the cell wall of root hairs mediated by bacterial activity at the locus of infection have been postulated earlier<sup>4</sup> and it is likely that loosening of the cell walls of *Rhizobium* by penicillin treatment might aid in successful entry of bacteria at many loci on the root system leading to better nodulation.

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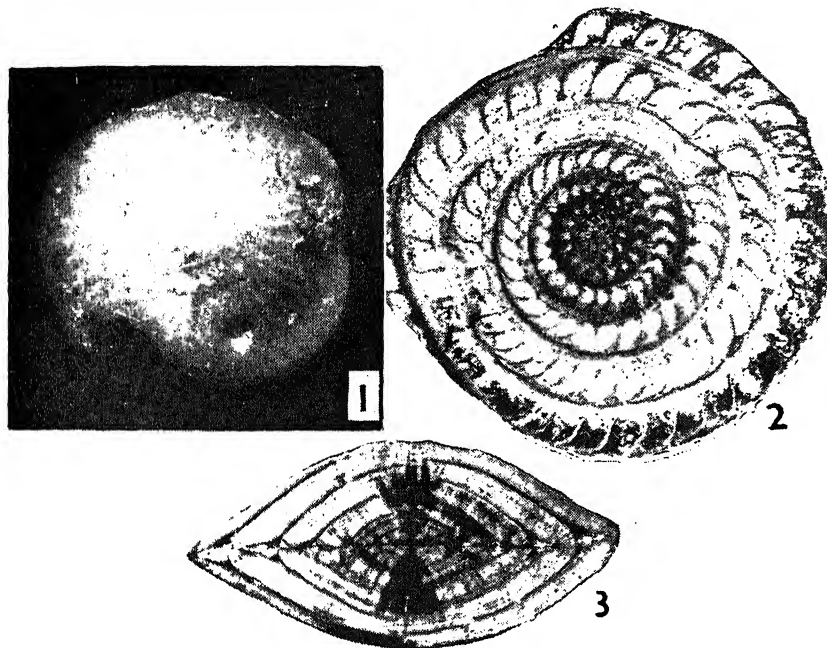
#### OCCURRENCE OF NUMMULITES PENGARONENSIS VERBEEK, AN INDO-PACIFIC LARGER FORAMINIFERA, IN THE MIDDLE EOCENE FULRA LIMESTONE OF CUTCH, GUJARAT

TERTIARY rocks ranging in age from Palaeocene to Pliocene are well exposed in the coastal strip of the mainland of Cutch, Gujarat<sup>1</sup>. Here, the Middle Eocene part of the succession is highly fossiliferous and contains abundant larger and smaller foraminifers. Larger foraminifers which constitute the dominant element in the fauna are represented by several stratigraphically significant genera including the most common Lower Tertiary genus *Nummulites* Lamarck. Since Sowerby's<sup>2</sup> publication on the Tertiary fossils from Cutch, several species of *Nummulites* have been reported by workers<sup>3-5</sup>

on the Eocene of Cutch. However, presence of *Nummulites pengaronensis* Verbeek which occurs frequently in the Fulra Limestone has not been recognised by any of them.

In the present study *N. pengaronensis* was observed in the samples of the Fulra Limestone collected from Lakhpat and Bermoti areas of Cutch. It is represented by both the microspheric and megalospheric generations. Megalospheric specimens are much more abundant than the microspheric ones. The species is characterized externally by the presence of thin, radial, gently curved septal filaments (Fig. 1) and the absence

The recorded stratigraphic range of the species is Late Middle Eocene to Oligocene. In Cutch *N. pengaronensis* is found to be restricted stratigraphically to the Fulra Limestone which has yielded characteristic Middle Eocene larger foraminifers such as *Alveolina elliptica* (Sowerby), *Assilina exponens* (Sowerby), *Nummulites maculatus* Nuttall, etc. The associated planktonic foraminifers which permit recognition of the *Orbulinoides beckmanni* and *Truncorotaloides rohri* Zones of Bolli in the Fulra Limestone indicate its age as Late Middle Eocene.



FIGS. 1-3. Fig. 1. *N. pengaronensis* Verbeek, external view of microspheric specimen, from Lakhpat, Cutch,  $\times 8$ . Fig. 2. *N. pengaronensis* Verbeek, equatorial section of microspheric specimen, from Lakhpat, Cutch,  $\times 10$ . Fig. 3. *N. pengaronensis* Verbeek, axial section of microspheric specimen, from Lakhpat, Cutch,  $\times 10$ .

of distinctly developed polar pustules and internally by the thick, curved septa in equatorial section (Fig. 2) and very narrow alar prolongations in axial section (Fig. 3). Our specimens compare satisfactorily with the original description and illustrations of the species provided by Verbeek<sup>6</sup> and also with the specimens of *N. pengaronensis* described and figured from the Kopili Formation of Eastern India<sup>7</sup>.

Originally described from the Eocene of Borneo, *N. pengaronensis* was later found to be widely distributed in the Indo-Pacific region and has been recorded from several other localities in the East Indies, the Central Pacific Islands, Burma, Eastern India and Pakistan. The present record extends the geographic distribution of the species to Cutch,

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# AN ABNORMAL CORPUSCLE OF STANNIUS IN THE CATFISH, *HETEROPNEUSTES* *FOSSILIS* (BLOCH)

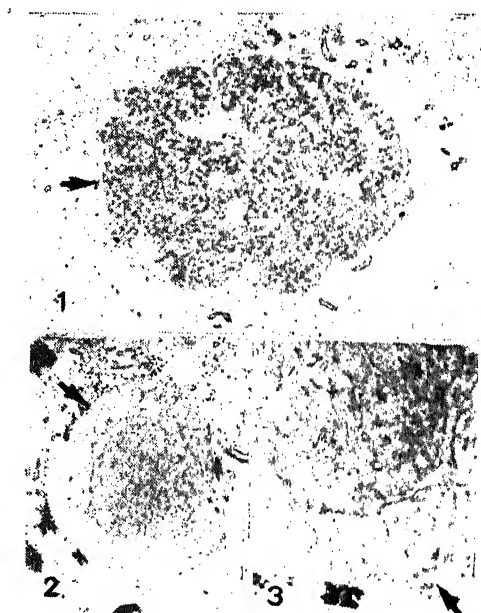
DURING the course of investigations on the implication of endocrine glands in the physiology of reproduction of the catfish, *Heteropneustes fossilis*, two abnormal conditions have been encountered and reported from this laboratory<sup>1-2</sup>. The present communication, which provides a brief description of an abnormal corpuscle of Stannius (CS) from the same species, forms the third report. The histomorphology of the CS has been studied in several fishes (see Krishnamurthy and Bern<sup>3</sup>), but no structural abnormalities seem to have been reported so far. This communication also argues against the concept of categorization of the CS of different species into one type on the basis of histological variations.

The CS of *H. fossilis* are round, oval or irregular, dull whitish bodies embedded partly or completely in the mesonephros (Fig. 1). Their number ranges between 1 and 4, and each corpuscle is invested by a connective tissue capsule from which many septa extend in-between the constituent cells.

The abnormal corpuscle observed in the mesonephros of a catfish seems to have been formed by aggregation of two different CS. The outer corpuscle appears cup-shaped, while the inner one is an oval body (Fig. 2). The former envelops about half of the latter. The outer corpuscle shows a well-organized tubular arrangement of the septa which divide the corpuscle into distinct, although incomplete, lobes (Figs. 2, 3). In preparations stained with aldehyde fuchsin (AF), the cells appear almost negative except at their basal parts located close to the connective tissue septum. In the outer corpuscular area, the nuclei are closely situated. The oval, inner corpuscle, on the contrary, does not exhibit any definite arrangement of the connective tissue septa. They are scattered throughout the CS and divide the corpuscle into irregular cell masses. The cells are deeply stained with AF, and the stainable material forms a distinct ring around each nucleus. The average number of nuclei in the inner corpuscle ( $25600 \pm 4190$  per  $\text{mm}^2$ ) is lower than the nuclear population of the outer corpuscle ( $36180 \pm 3380$  per  $\text{mm}^2$ ).

It seems plausible that the abnormal corpuscle reported here is formed by the union of two CS rather than mere occurrence of two distinct zones in one. This view receives support by the occurrence of marked differences between the two in the arrangement of the septa, disposition of the cells, their staining properties and the number of nuclei per unit area. A wide variety of intraspecific variations have been observed in the histology of

the CS of *H. fossilis* (unpublished data). In fact, all the 4 types of CS recognized by Krishnamurthy and Bern<sup>3</sup> among 28 species of fishes have been found among different specimens of *H. fossilis*. Different corpuscles even from the same specimen presented some minor histological differences. Ford<sup>4</sup> described regional structural differences in the CS of *Oncorhynchus gorbuscha*. The outer corpuscle corresponds with Type I, and the inner one with Type II recognized by Krishnamurthy and Bern<sup>3</sup>. In view of the remarkable differences between the two CS, and because several types of CS have been found in *H. fossilis*, it seems that the abnormal corpuscle probably represents two CS that migrated to lie apposed to each other during some ontogenic stage.



FIGS. 1-3. Fig. 1. Section of kidney showing a normal CS (arrow),  $\times 64$ . Fig. 2. Section of kidney showing abnormal CS (arrow). Note the outer cup-shaped, and inner oval CS,  $\times 48$ . Fig. 3. Section of the abnormal CS showing the outer corpuscle (arrow) with tubular arrangement of septa, while the inner corpuscle does not exhibit any definite arrangement of septa,  $\times 120$ .

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### FRONTAL ORGANS IN *RIVULOGAMMARUS SYRIACUS* CHEVREUX.

THERE has been a considerable amount of debate concerning the nomenclature and function of the frontal organs in crustacea. In Amphipods these organs have been described<sup>1-3</sup> with reservation in *Caprella* but Graber<sup>4</sup> claimed that these organs do not exist in *Gammarus*. During the course of studies on the cephalic neurosecretory system of *Rivulogammarus syriacus* the authors have investigated the frontal organs.

The frontal organs have been studied by using haematoxylin-eosin, Gomori techniques and histochemical tests. With these different methods it has been found that these are paired spherical bodies lying anterior to the protocerebrum. In the dorsal region they are just below the epidermis while in the middle region they are far away. They are connected to the medulla terminalis of the optic lobe by an oblique nerve (Fig. 1). Two kinds of structural elements are observed in the frontal organs. The first kind are the cells without neurosecretory material surrounded by small connective tissue cells; the second are small round colloidal concretions. The latter seem to be the secretory products of neurosecretory cells and have been transported through the axons (Fig. 2). The neurosecretory material in form of CHP-positive granules are also found along the course of axons which are coming from the medulla terminalis of the optic lobe. These neurosecretory materials have PAS-positive reaction.

There has been a considerable amount of discussion concerning the nomenclature and function of the organs of Bellonci and frontal organs. Claus<sup>5</sup> described the dorsal frontal organs and regarded them as sensory structures. Hanstrom<sup>6</sup> attributed secretory function to the frontal organs in *Tanymastix stagnalis* L. and *Polyartemia forcipata* and regarded them to be precursors of the X-organs of malacostracans. Elofsson<sup>7</sup>, working with different decapods, was able to show that the dorsal frontal organ, when present, was always part of the nauplius eye centre of the brain. The organs of Bellonci (sensory pore X-organs) are associated with the medulla terminalis in decapods (Knowles and

Carlisle<sup>8</sup>) and have been shown by Dahl<sup>9</sup> to be derived from neuroblasts of the medulla terminalis. Dahl<sup>10</sup> has concluded that the dorsal frontal organs and the organs of Bellonci represent phylogenetically quite independent structures because the dorsal frontal organs in the crustacea investigated so far, when present, are associated with the nauplius eye centre, while the organs of Bellonci are associated with the medulla terminalis. Menon<sup>11</sup>, Elofsson<sup>12</sup> and Lake<sup>13</sup> have attributed neurosecretory function to the organs of Bellonci (dorsal frontal organs) in anostraca.

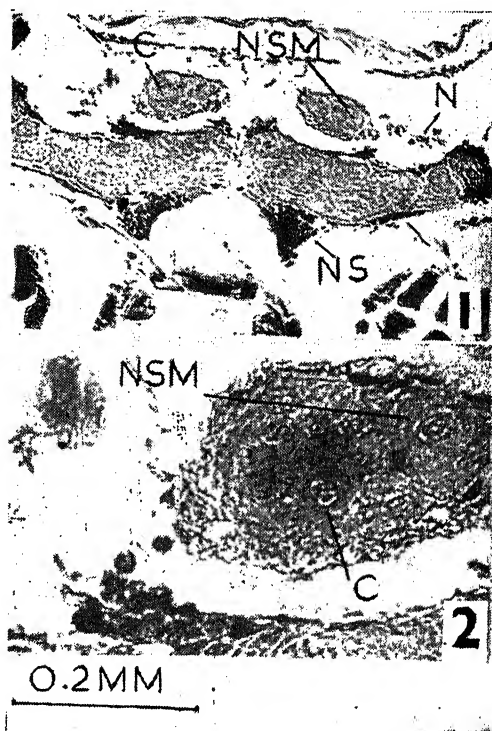


FIG. 1. H.S. of cephalic region of *Rivulogammarus syriacus*; showing frontal organs and supraoesophageal ganglion; Bouin; CHP; C—Cells; NSM—Neurosecretory material; N—Nerve; NS—Neurosecretory cells of the supraoesophageal ganglion.

FIG. 2. H.S. of frontal organ of *Rivulogammarus syriacus*; Bouin; CHP; C—Cells; NSM—Neurosecretory material.

In *Rivulogammarus syriacus* the paired frontal organs are quite distinct and are not innervated from the nauplius eye centre but from the medulla terminalis. These paired structures and their axons contain appreciable amount of CHP-positive granules. It appears that these structures are associated with both neurosecretory release, and perhaps, of neurosecretory material synthesis. These

functions are similar to those organs of Bellonci of other malacostracans, the frontal organs of the copepoda<sup>14</sup>, the X-organs of the copepods<sup>12</sup>, the sensory papilla X-organ of Cirriped larvae<sup>15</sup>, and finally to the dorsal frontal organs or X-organs of the Anostraca<sup>11-12-15</sup>. In the phyllopod crustaceans and copepods there is a sensory frontal organ often associated with a large neurosecretory cell. In amphipods it is connected to the medulla terminalis and associated with the release of neurosecretory material. In malacostracan crustacea the frontal organ is incorporated into the central nervous system where it forms an X-organ again revealing a connection between a originally epidermal glandular (and sensory) structure and a later neurosecretory centre. It is evident that the condition of frontal organs in *Rivulogammarus* is somewhat intermediate between the phyllopod crustaceans and copepods at one end and the malacostracan crustaceans at the other end.

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#### BILGRAMIA, A NEW GENUS OF HYPHOMYCETES

THE authors collected dead wood pieces bearing black fungal growth from Mount Abu and subsequently from Jodhpur. It was found that the fungus produces Sporodochia and the Conidia are phaeodictyosporous and catenulate. The conidia are connected by isthmi. Any cell of the conidia or isthmi may produce a chain of conidia with isthmi. The fungus resembles *Peyronelia*, *Alternaria* and *Sirodesmium* in some characters. Although the

present fungus is quite close to the genus *Peyronelia*, however, it cannot be accommodated in that genus as the conidia in *Peyronelia* are transversely septate phragmospores. Ciferri and Frago<sup>1</sup> had mentioned only transverse septa in their description of the type species of *Peyronelia* and hence Clements and Shear<sup>2</sup> have placed the genus *Peyronelia* in section Phragmosporae of the family Dematiaceae. Our fungus differs from *Alternaria* in having sporodochia, very short hyphae and in producing chains of conidia with isthmi from any cell of conidia or isthmi; and from *Sirodesmium* in the presence of isthmi. In view of these distinct morphological characters the fungus is being described as a new genus of Hyphomycetes. The name *Bilgramia* is proposed in honour of our teacher Prof. K. S. Bilgrami.

Since the fungus produces dark-coloured conidiophores; muriform, catenulate, dark conidia and very short hyphae, this new genus, *Bilgramia* is being placed along with the genus *Sirodesmium* in the family Dematiaceae, section Dictyosporae and the group Microneae.

*Bilgramia* gen. nov. Panwar, Purohit and Chouhan

Sporodochia sphaerica, non-stromatica; conidiophora simplicia, brevia, macronemetosa, determinata, brunnea. Conidia acro-murogena, phaeodictyospora; conidia catenata, per cellulam isthmam, concoloratam separata; isthmus elongatus cum 1-9 septis transversatis, conidiorum cellulae, vel isthmorum producentes paene omnes sporogenas cellulas.

Sporodochia spherical, non-stromatic; conidiophores unbranched, short, macronematous, determinate, brown; conidia acro-murogenous, phaeodictyosporous, conidia in a chain, separated by concolorous isthmus cells; isthmus elongated, with 1-9 transverse septa; sporogenous cells produced from almost all the cells of conidia or isthmi.

*Bilgramia indicum* gen. et sp. nov. Panwar, Purohit and Chouhan.

Fungus in ligno emortuo producit maculas fuscantes, saprophyticas, mycelium in substrato immersum, rarum, brunneum, arte septatum, 4-5.5  $\mu$  latum; sporodochia superficialia, sphaerica, non-stromatica; conidiophora non-ramosa, brunnea, arte septata, 20-25  $\times$  5.5  $\mu$ ; conidia maxime lurida, muriformia cum septis transversalibus 5-11 et 3-8 longitudinalibus, profunde, ad septa transversalia constricta, plerumque, obclavata raro globosa, vel cylindrica, paries verrucosus, catenulatus, conidia septata per isthmus; ulla cellula, isthmorum vel conidiorum producit conidiolum cum isthmis catena, conidia 27-60  $\times$  16-30  $\mu$  cellulae isthmi brunneae ad luridas, 1-9 transversales septatae, plerumque 5-septatae, paries laevis vel constrictus apud septum transversale, 8-45  $\times$  5-5.5  $\mu$ .

In ligno emortuo, collectum in Mt. Abu etiam Jodhpur, VIII, 1973.

Specimen depositum apud C.M.I., Kew, Herb. IMI 180026 typus and Botany Department, University of Jodhpur, Coll. J.U.M.L. 306.

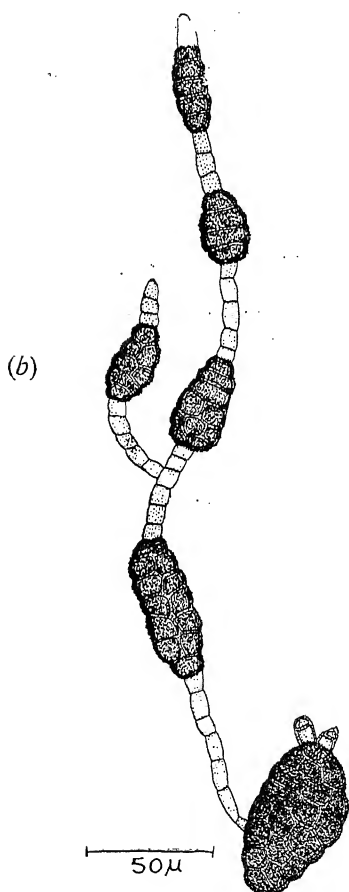
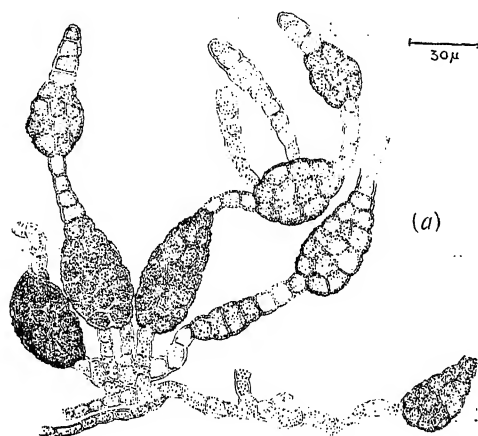


FIG. 1. (a) Sporodochium, conidiophores and conidia with isthmi; (b) Conidia with isthmi.

The fungus produces dark patches on dead wood, and is saprophytic. Mycelium immersed in the substratum, scanty, brown, closely septate,  $4-5.5 \mu$  wide; sporodochia superficial, spherical, non-stromatic; conidiophores unbranched, brown, closely septate  $20-25 \times 5.5 \mu$ ; conidia extremely dark-brown, muriform with  $5-11$  transverse and  $3-8$  longitudinal septa, deeply constricted at the transverse septa, usually obclavate, rarely globose or cylindrical, wall verrucose, catenulate, conidia separated by isthmi, any cell of isthmi or conidia producing a chain of conidia with isthmi, conidia  $27-60 \times 16-30 \mu$ ; isthmus cells brown to dark-brown,  $1-9$  transverse septate, usually  $5$ -septate, wall smooth or constricted at the transverse septa,  $8-45 \times 5-5.5 \mu$  (Fig. 1).

On dead wood, collected from Mount Abu and Jodhpur, August 1973.

Specimen deposited with C.M.I., Kew, Herb. IMI 180026 type and Botany Department, University of Jodhpur, Coll. J.U.M.L. 306.

The help received by Dr. M. B. Ellis is gratefully acknowledged. Thanks are also due to the Rev. Father William Barracos for the Latin diagnosis.

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#### NOTCH EXPRESSION IN RICE KERNELS

NOTCHED character in rice refers to a well-marked constriction on the ventral side of the kernel just above the embryo (Fig. 1). The trait in notched variety does not express in all the kernels, but it is present only in some of the grains in the panicle. Inter-varietal or even intra-varietal variation in the expression of this character has also been noted. Three dominant complementary genes along with an inhibitor have been suggested to control notching<sup>1</sup>.

In this situation, observation in the completely homozygous diploid (haploid chromosome complement doubled) possessing notching may provide some useful data. Such a study was possible when a haploid plant was obtained in an  $F_2$  of the cross between J.B.S. 820 (10-15% kernels notched) and AC. 1225 (unnotched kernels). The haploid plant was vegetatively multiplied in several hundred clones which resulted in production of some diploid grains. The present note gives an account of the degree and

expression of notching in some of the progenies of such grains.

Sixty grains were set in the haploid clones, out of which approximately half were notched. Five notched and five unnotched healthy grains were raised and studied. Plants raised from notched or unnotched grains had notching; but the percentage of notched kernels was not same. The highest percentage was found in Pl. No. 2 (89.8) and the lowest in Pl. No. 3 (61.3). The present study has revealed that the notching can be present at more than one point on the kernel. As stated earlier, notch generally appears on the ventral side slightly above the embryo. It may either be present at the apical portion of the kernel, on dorsal side of the kernel, on dorsal and ventral sides of the same kernel or at two places on the same side (Fig. 1). The occurrence of these new types of notching was not very frequent in the population and their proportion was also not definite. Notching was, however, not found on the flat portion of the kernel.

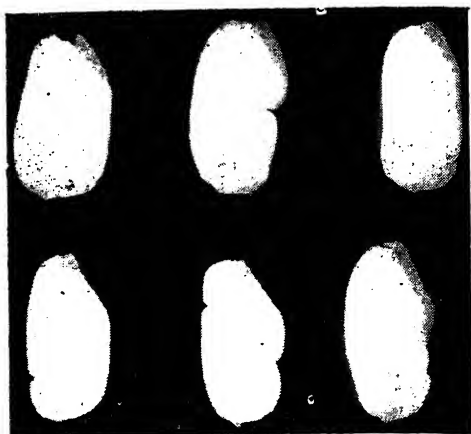


FIG. 1. Kernels exhibiting notching at different points.

The average higher expression in true diploid plants is in contrast to the maternal parent J.B.S. 820. Complete homozygosity in this case has been associated with high degree of expression of the character. More data are needed to verify whether this phenomenon is more common in the other varieties also. The notch expression has earlier been shown to be influenced by environment<sup>2</sup>. None of the plants, in fact, show cent per cent expression even in the completely homozygous plants.

Four unusual notch expressions indicate that the gene has variable expressivity. This has not been observed earlier.

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### THE EFFECT OF COLCHICINE ON SEX EXPRESSION IN MUSKMELON

Sex expression in muskmelon can be modified by exogenous application of auxins<sup>1,3,8,10</sup> gibberlins<sup>3,8,10</sup>, cytokinins<sup>3,4</sup>, morphactins<sup>5</sup> and ethrel<sup>6,7</sup> as well as by mineral nutrition<sup>2,9</sup> and environmental conditions such as light<sup>1,8</sup> and temperature<sup>3</sup>. The work described here was undertaken to isolate the effect of colchicine on sex expression in muskmelon.

Seeds of andro-monoecious variety of muskmelon (*Cucumis melo* L., variety, Hara Madhu) were sown in 30 metre long and 3 metre wide plots at a depth of 2 cm with spacing 80 cm between plants. They were later thinned out in a manner so that three replicates of ten plants were left in each plot. Plants were sprayed four times with aqueous solution of colchicine at 0.1, 1, 10 and 100 ppm since two true leaved stage at weekly intervals by hand automizer. Observations were recorded regarding the position and production of both staminate and hermaphrodite flowers. Sex ratio was calculated from this data and pollen sterility was isolated by staining them in iodine. During experiments minimum temperature was 25° C and maximum temperature was 38° C. Plants were grown in natural daylength of 11–13 hours.

The results are documented in Table I. The data pinpoint a marked tendency towards female sex expression in plants treated with colchicine. The first staminate flower differentiated on the 6.6, 6.9, 7.5, 9.0 node and the first hermaphrodite flower appeared on the 9.2, 8.0, 7.3, 15.1 in 0.1, 1, 10 and 100 ppm colchicine treatment respectively. Colchicine treatment increased the number of hermaphrodite flowers and decreased the number of staminate flowers over control (Table I) in muskmelon. The ratio of staminate/hermaphrodite flowers was 18.1:1, 12.2:1, 8.9:1 and 6.1:1 in 0.1, 1, 10, and 100 ppm colchicine application respectively. Colchicine application also induced pollen sterility which increased with increase in concentration in both staminate and hermaphrodite flowers (Table I). In case of hermaphrodite flowers 100% pollen sterility was noticed in 100 ppm treatment. Most of the flower buds showed various stages of



TABLE I

Effect of colchicine on sex expression in muskmelon (*Cucumis melo* L.)  
(Values are mean with  $\pm$ SE of mean)

Observations	Control	Colchicine concentration in ppm			
		0.1	1	10	100
Position of node bearing first staminate flower	5.1 $\pm$ 0.31	6.6 $\pm$ 0.75	6.9 $\pm$ 0.29	7.5 $\pm$ 0.38	9.0 $\pm$ 0.33
Staminate flowers	205 $\pm$ 6.2	200 $\pm$ 5.9	183 $\pm$ 5.1	161 $\pm$ 4.8	153 $\pm$ 4.6
Position of node bearing first hermaphrodite flower	14.8 $\pm$ 0.47	9.2 $\pm$ 0.38	8.0 $\pm$ 0.35	7.3 $\pm$ 0.11	15.1 $\pm$ 0.22*
Hermaphrodite flowers	10 $\pm$ 0.22	11 $\pm$ 0.19	15 $\pm$ 0.44	18 $\pm$ 0.36	25 $\pm$ 0.20
Ratio of staminate/hermaphrodite flowers	20.5 : 1	18.1 : 1	12.2 : 1	8.9 : 1	6.1 : 1
Pollen sterility % of staminate flowers	12	30	42	47	69
Pollen sterility % of hermaphrodite flowers	76	81	85	94	100

\* Not significant.

fusion and reduction in the size as well as in number of stamens. Some of the hermaphrodite flowers showed complete abortion of stamen, therefore, forming pistillate (female) flowers. The ovule fertility was not affected by colchicine treatment unlike pollen fertility. The plants treated with colchicine produced viable seeds. The viability of seeds was tested with T.C.C. test and 100% viability was recorded in various concentrations of colchicine like control.

The present observations clearly demonstrate that colchicine suppresses appearance as well as further development of staminate flowers and induces differentiation of hermaphrodite flowers at lower node on the main vine and also increases the number of hermaphrodite flowers in muskmelon. It is also an interesting point to mention here that colchicine has properties opposite to gibberellins and similar to those of auxins, cytokinins and morphactins in relation to sex expression. As gibberellins are known to stimulate male sex expression<sup>3,8</sup> auxins, cytokinins and morphactins are well known to induce femaleness in muskmelon<sup>1,3,4,5,8,10</sup>. Therefore it is possible that colchicine reverses the influence of endogenous gibberellin, acts through auxin metabolism or may have an independent activity which modifies the sex expression in muskmelon.

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#### MICRODISSECTION STUDIES ON THE DEVELOPING LEAF PRIMORDIA OF *PHOENIX SYLVESTRIS* L.

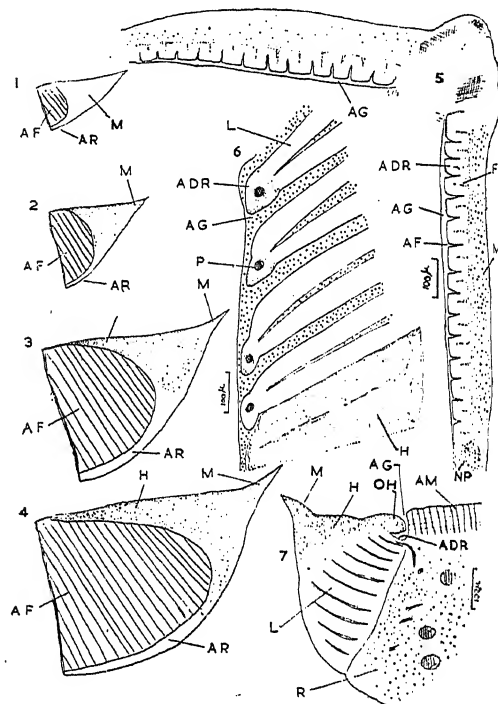
THE genus *Phoenix* is unique among the palms in its leaf morphology. This refers not only to the induplicate leaflets, but also to the presence of a peculiar tissue referred to as the Haut. The haut develops very early and covers the entire series of leaflets, grows along with the leaflets but dries up when the leaf unfolds. The haut had been the subject of much research since Von Mohl<sup>2</sup> published an account of leaf development in palms. According to his account the haut is part of the lamina and not a secondary product. Later workers expressed varied opinions on the nature of the haut.



Goebel<sup>1</sup> maintained the view that the haut is a secondary product of the lamina. According to his interpretation the marginal tissues grow over the leaflet primordia and fuse with the rachis, thus forming the haut. Recently, Periasamy<sup>5</sup> studied *Phoenix sylvestris* and concluded that the haut is a product of fusion of the 'adaxial ridges'\* and the marginal tissue. This view is essentially similar to that of Goebel<sup>1</sup>. However, Padmanabhan<sup>3</sup>, investigating the leaf development in *Phoenix*, concluded that the haut is a part of the lamina and not a fusion product of the adaxial ridges. The present reinvestigation was initially undertaken to verify the role of proliferations from the adaxial ridges during the ontogeny of the haut as mentioned by Periasamy<sup>5</sup>. Incidentally, this investigation revealed new aspects which were hitherto unknown.

The technique of microdissection of very young isolated laminal primordia was adopted in this study. This method proved to be useful in solving many of the problems that have arisen in studies mainly depending on sectioned leaf material. The youngest leaf primordium dissected out of the shoot bud measured 50 microns from the base of the rachis to the tip. This primordium had wholly unplicate lamina measuring 10 microns at its broadest area. Plications were just initiated in primordia measuring 150 microns high. At the back of the terminal part of the lamina seven abaxial ridges were already formed on either side. Each ridge was about 6 microns broad. A primordium measuring 150 microns high had 15 abaxial ridges developed on each side. A close examination of the adaxial side of the lamina indicated the formation of short adaxial furrows and ridges all along (Fig. 5). But these were not visible under the low powers of the microscope. Gentle squashing and isolation of lamina yielded material which was thin enough for re-examination under high power. Such a study indicated that the adaxial furrows are regularly formed and that they get more or less occluded in the adaxial groove that forms along with the origin of plications and gets deeper as it grows older. In the primordia measuring 600 microns high the adaxial ridges were not visible but dissections using microscalpels revealed them. This was made possible by dissecting off the rachis tissue adjoining the adaxial groove. The adaxial ridges were still short and stumpy indicating that they failed to keep pace with the abaxial growth. But microneedles inserted into the abaxial ridges revealed that the adaxial furrows have kept growing backwards in contrast to the adaxial ridges. This means that the adaxial furrow grows into the laminal tissue and therefore becoming internal for the major part (Figs. 1-4). They

open out only in the adaxial groove between the short stunted adaxial ridges. Free adaxial ridges were clearly seen in paradermal sections of the haut tissue (Fig. 6) confirming the conclusions of the microdissection studies. A re-examination of transverse sections of the leaf primordia at various stages of development indicated that the short adaxial ridges and furrows could be made out adjoining the rachis and in the adaxial groove (Fig. 7). The haut is often seen overhanging the adaxial ridge or furrow.



FIGS. 1-7. *Phoenix sylvestris* L. Figs. 1-4. Diagrams illustrating the origin of the haut by abaxial overgrowth and ingrowth of adaxial furrow while there is no growth in the adaxial ridges. Fig. 5. Camera lucida sketch of very young plications seen in the isolated lamina after gentle squashing of the central axis of the young leaf primordium. Fig. 6. Part of paradermal section of the haut tissue of a leaf primordium measuring 8 mm. Note the free adaxial ridges in the adaxial groove and the haut tissue covering the plications below, and located over the adaxial ridges and furrows. Fig. 7. Transverse section of the leaf primordium showing the rachis, the lamina, the adaxial haut tissue which overhangs the adaxial ridge. AF—Adaxial furrow; AG—adaxial groove; ADR—Adaxial ridge; AM—Adaxial meristem; AR—Abaxial ridge; H—Haut; L—Leaflet; M—Marginal tissue; NP—Nonplicate young region of lamina; OH—overhanging haut tissue; P—primary vascular bundle in adaxial ridge.)

The results of this investigation solve several problems about the haut. First of all, it has been established that adaxial furrows and ridges are present as such and not fused even in the older primordia. The earlier claim that the adaxial furrows and ridges are seen only in the region of newly forming plications and become fused in older regions<sup>5</sup> is incorrect. Secondly, the adaxial furrows which were thought to be totally internal<sup>3</sup> have been shown to open out through the short slits between the adaxial ridges occluded in the adaxial groove. Lastly, the mechanism of haut development is shown to be the result of rapid abaxial growth, the lack of growth in the adaxial ridges and the inward growth of the adaxial furrow keeping pace with the abaxial growth. Thus, the laminal tissue lying above the inwardly extended adaxial furrow becomes the haut which naturally binds the leaflets.

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\* See Periasamy (1962) for details of formation of furrows and ridges during the early ontogeny of the plications.

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#### INHERITANCE OF PIGMENTATION IN AN INTERSPECIFIC CROSS BETWEEN *ELEUSINE* *CORACANA* AND *E. AFRICANA*

*Eleusine coracana* (L.) Gaertn., commonly known as Finger millet, is widely cultivated for its grain in East Africa and South Asia. The grain is mostly used for preparing cakes, porridge and beer. *E. africana* Kennedy-O'Byrne is a wayside weed and is distributed in tropical and South Africa. It is reported to be a contaminant in the cultivation of this millet especially in Uganda<sup>1</sup>. These two species are tetraploid with  $2n=36$  chromosomes. Hiremath (1973)<sup>2</sup> has analysed the genome structure of *E. coracana* and has shown that the genomes of these two species are similar. It is also suggested that *E. coracana* might have originated through selection and further cultivation from *E. africana*. The interspecific hybrids and their progenies were found to be completely fertile.

In *E. coracana* there are several varieties which are purple pigmented. The pigmentation is of anthocyanin type. The presence and distribution of this pigmentation serves as a useful guide in the broad grouping of varieties. *E. africana* is a green pigmented species. The present communication deals with the inheritance of pigmentation in an interspecific cross between *E. coracana* and *E. africana*.

*E. coracana* P.I. 324 is a purple pigmented type. The pigmentation is normally distributed over midrib, leaf sheath, node, internode and glumes. This variety was crossed with *E. africana* P.I. 399 a green pigmented plant during 1968. The  $F_1$  was raised in 1969. The  $F_2$  population consisting of 416 individuals obtained by selfing  $F_1$  was studied in 1970. The performance of 100 randomly selected  $F_3$ , selfed from  $F_2$ , was recorded in 1971. The segregations observed in  $F_2$  and  $F_3$  generations are given in Tables I and II.

TABLE I

The mode of inheritance of pigmentation in the  $F_2$  of the cross P.I. 324 *E. coracana* × P.I. 399 *E. africana*

Material	Number of plants			$X^2$	P-value
	Purple	Green	Total		
$F_2$ Observed	315	101	416	0.11	0.70
Expected (3:1)	312	104	416	..	..

TABLE II

Segregation of pigmentation in the  $F_3$  of the cross P.I. 324 *E. coracana* × P.I. 399 *E. africana*

Material	Number of families			Total	$X^2$	P-value
	Homo-zygous purple	Hetero-zygous (3:1)	Homo-zygous green			
$F_3$ Observed	23	55	22	100	1.02	0.5
Expected	25	50	25	100	..	..

The  $F_1$  is purple pigmented. Out of 416  $F_2$  plants 315 are purple pigmented and 101 are green. This gives a good fit to a monohybrid ratio of 3 purple : 1 green ( $X^2=0.11$  and P-value 0.70). Thus purple pigmentation is controlled by a single dominant gene (P) and green colour (p) behaves as a recessive. This was further confirmed from  $F_3$  data (Table II). The  $F_3$  segregation showed a good fit to a ratio of 1 homozygous purple : 2 heterozygous : 1 homozygous green ( $X^2=1.02$  and P-value 0.5). An analysis of the heterozygous ones in this generation showed to segregate in a ratio of 3 purple : 1 green. The  $F_2$  and  $F_3$  data, therefore, prove conclusively that the purple

pigmentation of *E. coracana* is dominant over green colour (a recessive character) of *E. africana* and is inherited in accordance with monohybrid ratio.

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### ENDOGENOUS GIBBERELLIN AND INHIBITOR CONTENTS IN THE SHOOT APICES OF TOMATO PLANTS

A DIURNAL variation in the auxin content of tomato leaves was demonstrated by Went<sup>1</sup>, the values during the night being less than the day values. Bonga and Clark<sup>2</sup> observed variations in the IAA content associated with seasonal changes in the sapwood of balsam fir and they associated the fluctuations with seasonal cambial activity. A clear rhythm in the content of IAA was also observed in the young unfolding leaves of *Hibiscus esculentus*, L. and also between young and old leaves of *Piper betel* L.<sup>3</sup>. However, such a diurnal variation was not studied for the endogenous gibberellin content ( $GA_3$ ) and growth inhibitors like abscisic acid (ABA). Since growth is generally controlled not only by auxins, but also by gibberellins and inhibitors, i.e., by an interplay of promoters and inhibitors<sup>4</sup>, it was intended to determine the activity of gibberellins ( $GA_3$ ) and inhibitors (ABA) in the shoot apices of tomato plants grown under natural conditions at intervals of six hours during a 24-hour cycle, using the rice second leaf sheath bioassay test. The presence of ABA-like inhibitors in the gibberellin bioassay was shown by Barnes and Light<sup>5</sup> and Rajagopal and Rao<sup>6</sup>.

The gibberellin-like substances were extracted in 70% acetone<sup>7</sup> from 2.0 g shoot apices (shoot apex including two young just-open leaves) of 45 day old tomato plants at intervals of six hours on two successive days beginning at 6 A.M. After evaporation the aqueous fraction was acidified and extracted thrice with ethylacetate and the pooled extract was concentrated to a small volume and strip loaded on Whatman No. 1 chromatography paper and developed in isopropanol : ammonia : water (10 : 1 : 1). The dried chromatograms were cut into ten equal strips and each strip was

eluted overnight in 3.0 ml of ethylacetate in a test-tube, and then the eluate was evaporated to dryness. The residue was dissolved in 3.0 ml of glass distilled water and the biological activity was determined using the rice second leaf sheath bioassay test<sup>8,9</sup>.

The  $R_f$ s corresponding to authentic  $GA_3$  (0.4-0.5) where growth promotion of the leaf sheath occurred (over control) and that corresponding to authentic ABA (0.6-0.7) where growth inhibition occurred are extrapolated with a standard curve prepared with different concentrations of authentic  $GA_3$  and ABA respectively. Thus, the growth promotion and inhibition were converted into  $\mu g$  equivalents of  $GA_3$  and ABA respectively. Although there was both growth promotion and inhibition at other  $R_f$ s, it was not marked and so is not considered in the present study.

It is evident from Fig. 1 that ABA was at a high level during early part of the day and declined by night as indicated on both days.  $GA_3$  exhibited a gradual increase from 6 to 18 hours on both days, although the actual values were different for the two days, followed by a fall during midnight. A tendency for the  $GA_3$  level to increase whenever ABA declined was evident from the values between 12 to 18 hours of both days, i.e., a rapid fall in ABA coincided with a steady increase in  $GA_3$ . The low level of  $GA_3$  might be attributed to the counteracting effect of ABA on the gibberellin biosynthesis<sup>10</sup> and/or its activity<sup>11</sup>.

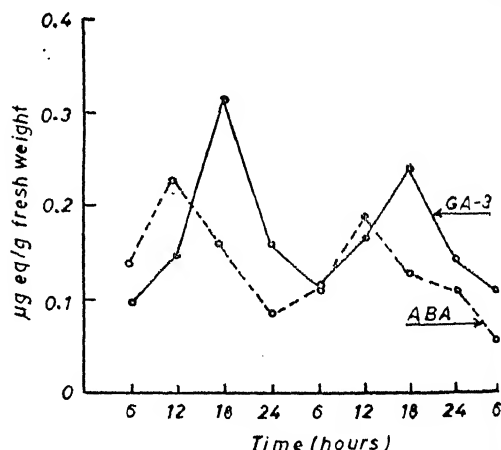


FIG. 1. Changes in the contents of  $GA_3$  and ABA ( $\mu g$  equivalents) in the shoot apices of tomato plants during a 24-hour cycle. Values are means of two determinations.

According to Wright<sup>12</sup> a system exists in the plants where gibberellins are synthesized mainly at night and inhibitor in the day. The relatively high ABA content at 12 hours on both days

supports the above view but the low  $GA_3$  content during midnight does not lend support. The high level of  $GA_3$  was evident only during early part of the night. Auxin content in tomato plant was reported to be more during the day than the night values<sup>1</sup>. Thus, the present study indicates a peak activity of  $GA_3$  at 18 hours and ABA at 12 hours and secondly an inverse relationship between the two (i.e., when ABA activity increased,  $GA$  activity declined) clearly between 12 and 18 hours on both days, but fails to indicate consistent diurnal rhythms. The rhythmic change between the two might be controlled not only by the environmental factors like light and temperature but also by the growth stage of the plant at which the analysis was made. Further investigation in this line would be interesting in clearly understanding the diurnal changes in the level of growth regulators, both promoters and inhibitors, at different stages of plant growth.

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## CULTIVATION OF ISOLATED LEAF CELLS OF *DOLICHOS LAB-LAB*

MUCH interest has recently been shown in the sterile culture of isolated cells. Remarkable success had already been achieved by Steward and his collaborators who were able to induce growth, cell division, and organization in isolated carrot cells<sup>1</sup>. Subsequently, there have been reports of successful isolation and cultivation of mesophyll cells of tobacco<sup>2</sup>. It has also been shown that mesophyll protoplasts, divested of their walls by enzyme treatment, regenerate their walls and divide to form a callus which, in turn, produces plantlets<sup>3</sup>. While these studies provide an insight into the capacity of mesophyll cells to de-differentiate, much interest lies in promoting their growth without callusing and loss of chlorophyll. The present authors studied this possibility in the leaf cells of *Dolichos lab-lab* with a view to perpetuate the progeny of these cells as functional photosynthetic units.

The leaf cells were isolated by adopting the grinding technique of Gnanam and Kulandaivelu<sup>1</sup>. About 10 grams of fresh leaves were surface sterilised by treating with chlorine water for three minutes, and thoroughly washed with sterile water. The leaves were mildly ground in cold sucrose phosphate buffer (pH 7.8), and the homogenate filtered through a double layer of muslin cloth to remove debris. The filtrate was centrifuged at 500 g for 1 min. The pellet was resuspended in cold buffer, and shaken thoroughly to distribute the cells uniformly. The concentration of cells in the suspension was determined by counting with a haemocytometer. This enabled inoculation of known number of cells in each culture flask.

The culture medium consisted of White's<sup>4</sup> minerals, sucrose (40 g/l), thiamin hydrochloride (0.1 mg/l), nicotinic acid (0.5 mg/l), pyridoxine hydrochloride (0.5 mg/l), myo-inositol (90 mg/l), 2,4-D (0.02 mg/l), and kinetin (0.2 mg/l). The pH was adjusted to 5.6. The medium was dispensed in 250 ml conical flasks (70 ml/flask), and sterilised by autoclaving. In each flask 5 ml of the cell suspension (containing  $10^6$  cells per ml) was inoculated, and the flasks shaken continuously over a gyrorotatory shaker. Illumination was provided by a battery of fluorescent lamps delivering 2,500 lux at the level of culture flasks. The temperature was maintained at  $24^\circ\text{C} \pm 1^\circ\text{C}$ .

The cultures were maintained for 90 days. Samples were withdrawn every 5 days from the culture flasks, and examined under the microscope. The cells kept normal appearance and were green throughout. Cell counts indicated that cell divisions had occurred in the cultures. After 60 days,

there was 30–40% increase in cell number, in different culture flasks. Microscopic examination revealed that the isolated mesophyll cells were dividing, and forming colonies of 10–20 cells (Figs. 1, 2). Aggregates of 2 or 3 cells were more frequent than colonies. But the majority of cells remained single. Cells usually divided by a transverse wall, and the chloroplasts of the parent cells were equally shared by the daughter cells (Fig. 3).



FIGS. 1–3. Leaf cells of *Dolichos lab-lab* from liquid suspension cultures. Fig. 1. General low power view of cells withdrawn from a 30-day culture,  $\times 60$ . Fig. 2. A colony of small cells with chloroplasts,  $\times 125$ . Fig. 3. Enlarged view of a divided mesophyll cell, note the transverse cross wall,  $\times 700$ .

A low level of auxin (0.02 mg/l of 2,4-D) sustained cell growth without callus formation. The cells remained green even after division. These results indicate that the mesophyll cells retain the capacity to divide, and keep green in a heterotrophic medium. While Takebe *et al.* were able to induce callus from tobacco leaf cells, the present work on *Dolichos lab-lab* has shown that prevention of callusing was essential for the cells to maintain their differentiated state. The concentration of auxin in the medium is critical in this respect since higher auxin levels (0.2 mg/l) invariably induced de-differentiation. The culture of dividing mesophyll cells could form excellent starting point for 'training' the cells to thrive on autotrophic media.

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Madurai 625021, May 9, 1974.

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#### AIR CAVITIES IN THE CULMS OF GENUS

THE presence of air cavities in the culms of grasses is rather uncommon. Mention of their presence in a few species of grasses like *Oryza sativa*, *Andropogon hexandra* and species of *Stipa* and *Sacciolepis* has been made by Metcalf<sup>1</sup> and Gould<sup>2</sup>. These cavities are mostly found in the culms of those grasses which grow in aquatic or marshy habitats.

During a recent course of studies on the anatomy of Kashmir grasses, the authors came across a conspicuous character in the transverse sections of culms of some species of genus *Poa*. A survey of literature on the anatomy of grasses shows that this character in the genus *Poa* has not been recorded so far. The present study is based on six species of *Poa*. These species are: *P. L.*; *P. pratensis* L.; *P. palustris* L.; *P. angustifolia* L.; *P. alpina* L. and *P. bulbosa* L. These air cavities are of different shapes in different species and are continuous longitudinally in the culms. In all the species studied, they are distributed at the periphery, between the vascular bundles. The exact location varies in different species. In some they may be just beneath the epidermis while in others a little below, preceded by a few layers of cells. These cavities are illustrated in a representative transverse section of the culm of *Poa pratensis* (Fig. 1). In this case these cavities are situated just beneath the epidermis, between the large and small vascular bundles and are semicircular in outline. It is interesting to note that none of the grasses studied for this character grows in aquatic or marshy habitats. To check the presence of these cavities, materials from 10 different habitats, especially those having dry habitats, were studied with positive results. Sections taken from the different levels of the culm showed the stability of this character. Sections taken immediately below the inflorescence showed

cavities to be smaller in size but their shape remained constant.

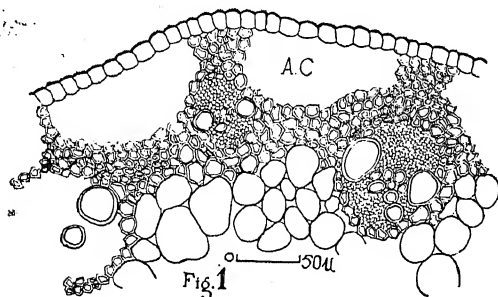


FIG. 1. Transverse section of the culm of *Poa pratensis* L. A.C., Air Cavity.

Keeping in view the stable occurrence and different shapes of these cavities in different species of genus *Poa*, this character can possibly be utilized in relation to the taxonomy of this large genus. Metcalf<sup>1</sup>, however, does not consider the culm anatomy a helpful taxonomic character. On the contrary the presence of these cavities in the rhizomes of bamboos has been used as a taxonomic character by McClure<sup>3</sup>.

Botany Department,  
University of Kashmir,  
Srinagar 190006, May 11, 1974.

BIMAL MISRI.  
KIRTEE K. KOUL.

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### THREE FUNGI FROM GROUNDNUT LEAF SURFACE

WHILE screening leaf surface flora of groundnut crop several species of fungi have been isolated and cultivated on PDA (Potato Dextrose Agar) slopes. Some of them were found responsible for causing leaf spots. One of the leaf spots on PDA plate gave rise to a culture which was identified as a species of *Alternaria*. Its morphological characteristics were compared with several species of *Alternaria* reported earlier by different workers like Neergard (1945), Deshpande and Rajderkar (1964) and Raghunath (1964) and it was found that it differed from them. Therefore it is reported here as a new species.

*Alternaria arachidis* sp. nov.

Coloniae in PDA efformantes massas lanatas, profuse crescentes aeree, ablae in juvenili conditione, postea nigrescentes, maculae foliorum brunneae,

irregulares, marginales, zona luteola circumdatae, 0.3-1.1 × 0.2-0.6 mm; conidiophora brunnea, brevia, septata, nec fuscata, 6.15-14.25 × 3.28-4.51 μ, conidia muriformia, brunnea, 6-10 cellularis, catenulata, ad apices attenuata, producentia rostrum breve 10.4-26.8 × 10.4-20.0 μ, rostro incluso 8.2-24.6 × 10.4-20.0 μ rostro excluso.

*Alternaria arachidis* sp. nov.

Colony on PDA a cottony mass, aerial growth profuse, white while young, later blackish, leaf spot on groundnut brown, irregular, marginal with yellowish halo round it, 0.3-1.1 × 0.2-0.6 mm conidiophores brown, short, septate, unbranched, length 6.15-14.25 × 3.28-4.51 μ, conidia muriform, brown, 6-10 celled, catenulate, attenuated at apex, producing a short beak, 10.46-26.8 × 10.4-20.0 μ with beak, and 8.2-24.6 × 10.4-20.0 μ without beak.

Number of leaf spots on the crop was numerous in the field and so it can be suggested here that the fungus may become a major pathogen of the groundnut crop under favourable conditions of weather.

The brown spot caused by the species of *Alternaria* on leaves of groundnut plant was, on further observation, found to harbour black fruit bodies of another fungus. Morphology of this fungus was studied and on comparison it was found that it resembled species of *Aposphaeria*. It was further compared with six species of *Aposphaeria* already reported and on the basis of its differences with them it is reported here as a new species.

*Aposphaeria arachidis* sp. nov.

Pycnidia insidentia maculis *Alternariae*, superficialia, completa disjuncta, papilla praesente et brevi, ostiolo definito, nequaquam in subiculo nec infra fastigata, rotunda vel ovalia, nigra, 46.8-72.8 × 20.8-67.8 μ.

Hyphae furcatae, septatae, hyalinae; conidiophora brevia, nec furcata, nec septata, hyalina, 1.9-2.3 × 1.0-1.2 μ, conidia unicellularia, hyalina, unius sortis, exogena, ellipsoidea vel ovoidea, appendicibus nullis, solitaria, 6.1-6.9 × 2.1-2.3 μ.

*Aposphaeria arachidis* sp. nov.

Pycnidia on *Alternaria* leaf spot of groundnut, superficial, complete, separate, papilla present and short with definite ostiole, not on subiculum, not tapering below, round to oval, black, 46.8-72.8 × 20.8-67.8 μ.

Hyphae branched, septate, hyaline; conidiophores short, unbranched, nonseptate, hyaline, 1.9-2.3 × 1.0-1.2 μ; conidia one celled, hyaline, of one kind, exogenous, ellipsoid to ovoid, without appendages, solitary, 6.1-6.9 × 2.1-2.3 μ.

Another fungus which produced fruit bodies on the necrotic brown spot of groundnut caused by *A. arachidis* was found to be *Phyllosticta* species. Its morphological characters resemble *P. jasminorum* Tognini, and therefore it is tentatively placed in the same species. It grew on the leaf spot as a saprophyte.

The author is thankful to Dr. S. T. Tilak, Reader in Botany, Marathwada University, Aurangabad, for his helpful guidance and encouragement.

Department of Botany, R. L. KULKARNI,  
Pratibha Niketan Mahavidyalaya,  
Nanded (S.C.R.) 431601, April 30, 1974.

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#### FLORAL ANATOMY OF *BROUSSONETIA* *PAPYRIFERA* VENT. (MORACEAE) WITH SPECIAL REFERENCE TO ITS GYNOCIDIUM

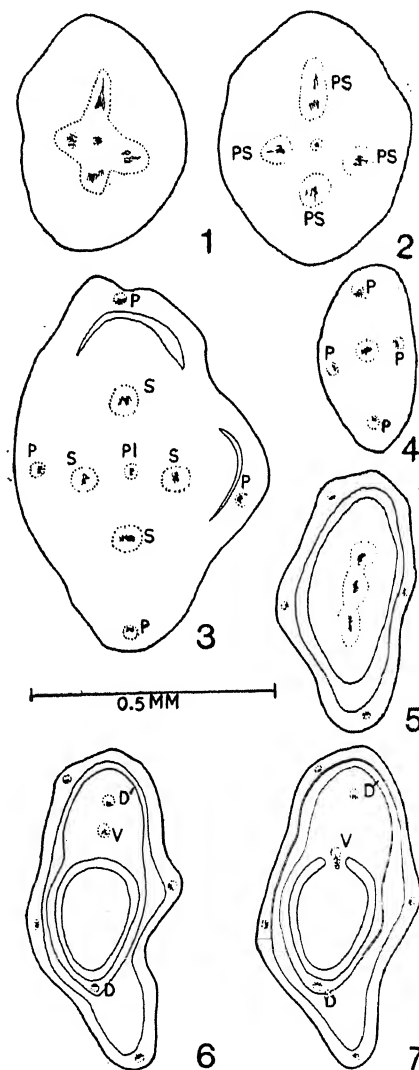
BECHTEL<sup>1</sup>, on the basis of his study of floral anatomy of some Urticales, concluded that in this group the bicarpellary condition of the gynoecium is derived from polycarpellary condition and by suppression of one of the carpels the bicarpellary gynoecium is becoming unicarpellary. The present investigation on the floral vasculature of *Broussonetia papyrifera* Vent. (Moraceae) has been undertaken with a view to assess the nature of its gynoecium.

The flowers for this study were collected from Hastinapur (Meerut), fixed in F.A.A., embedded in paraffin following usual methods and sectioned at 8–10 microns. They were stained with crystal violet-erythrosin combination.

*Broussonetia papyrifera* is a dioecious or occasionally monoecious<sup>2</sup> tree. The flowers are minute, regular and unisexual; the male flowers are borne in cylindrical spikes and the female in compact heads. The perianth is four lobed in both staminate and pistillate flowers. The stamens are opposite to and inserted at the base of the perianth. The gynoecium is apparently monocarpellary with one-celled superior ovary having a single pendulous ovule. The style is filiform and simple. A small pistillode is also present in staminate flower.

A continuous vascular cylinder constitutes the vascular supply of a flower. In male flower it breaks in four strands leaving a small mass of vascular tissue in the centre (Figs. 1, 2). The latter constitutes the vascular supply of the pistil-

lude (PI). The former which are the conjoint perianth stamen traces (PS) tangentially split into two each. The outer (P) eventually supplies a perianth lobe and the inner (S) to the accompanying stamen (Fig. 3).



Figs. 1–7. *Broussonetia papyrifera*. Figs. 1–3. Cross-sections of staminate flower at three different levels from base upward. Figs. 4–7. Cross-sections of pistillate flower from base upward. D = dorsal bundle of fertile carpel; D' = dorsal bundle of suppressed carpel; P = perianth trace; PI = vascular supply of pistillode; PS = conjoint perianth-stamen trace; S = stamen trace; V = ventral strand of carpel.

The vascular cylinder supplying the female flower first gives off four traces for the perianth in two

successive whorls, and then differentiate into three traces (D, V, D') (Figs. 4-6). Two of the latter are the dorsal carpel traces (D, D') which traverses into the style and extend upto the base of the stigma. The third trace (V) is eventually used up in supplying the pendulous ovule (Fig. 7).

Bechtel<sup>1</sup> reported three traced perianth in Moraceae and corresponds it to the palmate venation of the foliage leaf. He considers it a sign of primitiveness. Contrary to this, the perianth of *Broussonetia* is single traced though node is triacunar three traced in this species.

The presence of two dorsal bundles in the gynoeceium of *Broussonetia* suggests the presence

of two carpels of which one is suppressed while the other is fertile and bears a single ovule. The trace which supplies the ovule (V) seems to be the fusion product of the ventral bundles of both fertile and suppressed carpels.

The presence of pistillode in staminate flower indicates that unisexual condition in this species is derived from bisexual one.

School of Plant Morphology,  
Meerut College,  
Meerut, May 6, 1974.

V. SINGH.  
P. K. DUBLISH.

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## SHORT SCIENTIFIC NOTES

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### *Aspergillus flavus* Link., A Fungal Parasite on the Leaf-Eating Caterpillar, *Lymantria obfuscata* Walk.

*Aspergillus flavus* Link. is a saprophytic air-borne mould which grows on a variety of substrates such as grains, dairy products, vegetables, fruits, leather, and textile materials. The fungus is also known to infect a wide range of insects<sup>1-5</sup>. This pathogenic action is attributed to the toxic action of the fungus<sup>3</sup>.

During a survey of the pests of cacao occurring in India which has been undertaken by the Central Plantation Crops Research Institute, Regional Station, Vittal, a brownish hairy caterpillar was found to be damaging severely the tender foliage of cacao. This pest has been identified as *Lymantria obfuscata* Walker (Lep., Lymantriidae) by the Commonwealth Institute of Entomology, London<sup>6</sup>. It occurs throughout the year, but its population increases considerably after the rainy season. During the rainy season, a few of the caterpillars collected from the field were found to be infected by the fungus *Aspergillus flavus* Link. (Ex. Fr. CMI 175413). The affected caterpillars first developed yellowish-white patches on the body, later they became sluggish and stopped feeding. In advanced stages of infection, whitish and greenish-olive coloured conidiophores of the fungus covered the entire body of the caterpillar which progressively become shrunken and mummified. A pure culture of the fungus was obtained from the infected insect in Czapek's agar and it sporulated within four days.

The pathogenicity of the fungus was tested by dusting its conidia on the different development stages of *Lymantria obfuscata*, viz., egg, caterpillar, pupa and adult, and incubating them at 30-32° C. Healthy caterpillars were also fed with cacao leaves sprayed with spore suspension of the fungus. More than three-fourth of these caterpillars under both the treatments developed the symptoms given above and died within 3-4 days. The re-isolated fungus from infected caterpillars was found to be similar to the original culture.

This is the first record of "the fungus on *Lymantria obfuscata* Walk.

The authors are grateful to the Director, Commonwealth Institute of Entomology, London, for identifying the pest, and the Director, Commonwealth Mycological Institute, London, for identifying the fungus.

Central Plantation Crops R. RADHAKRISHNAN NAIR.  
Research Institute, T. PREMKUMAR.  
Regional Station,  
Vittal 574 243. S.K. Dt.,  
Karnataka, September 22, 1973.

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**Additions to the Host Records of the Root-Knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood**

Roots of a number of wild and cultivated plants belonging to different families were examined for infestation by root-knot nematodes in western Uttar Pradesh. Mature females were dissected out from the galled tissue of roots and their perineal patterns were cut; and stained with hot 0.01% acid fuchsin in lactophenol. The close study of these perineal patterns (Whitehead<sup>7</sup>) revealed that the species involved was *Meloidogyne incognita* (Kofoid and White) Chitwood. Some of these plants were found to be new hosts which have not been reported earlier (Goodey *et al.*<sup>3</sup>, Davidson and Townshend<sup>2</sup>, Potter *et al.*<sup>4</sup>, Whitehead<sup>8</sup>, Sitaramaiah *et al.*<sup>6</sup>, Roy<sup>5</sup>, Alam *et al.*<sup>1</sup>). The list of plant species along with the host response is given in Table I. The host response was rated as: 1 = light infection, 2 = moderate infection, 3 = heavy infection and 4 = severe infection. Size of root-galls, ranging from small (S), medium (M) to large (L) was also noted.

TABLE I

*Response of plants to the attack of the root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood, with some new host records*

Hosts	Size of root-galls	Reaction of host	Locality
<i>Achyranthes aspera</i> L.	M, S	2	Aligarh
<i>Ageratum conyzoides</i> L.	S	1	"
<i>Amaranthus gracilis</i> Desf.*	S	1	"
<i>Arundo donax</i> L.*	S	1	"
<i>Brassica oleracea</i> var. caulocarpa L.	S, M	1	"
<i>Calendula officinalis</i> L.	M	2	Agra
<i>Celosia argentea</i> L.**	L	4	Bulandshahr
<i>Celosia cristata</i> L.	L, M	3	Aligarh
<i>Chenopodium amaranticolor</i> Coste and Reyn.*	S	1	"
<i>Chenopodium ambrosioides</i> L.**	S	1	"
<i>Chenopodium murale</i> L.**	S, M	2	"
<i>Coleus blumei</i> Benth.**	M, S	3	Bulandshahr
<i>Corchorus capsularis</i> L.	M, S	2	Agra
<i>Eclipta alba</i> (L.) Hassk.	S, M	2	Bulandshahr
<i>Eleusine indica</i> Gaertn.	S	1	Aligarh
<i>Petunia hybrida</i> Vilm.	M, L	3	Bulandshahr
<i>Rumex dentatus</i> L.	S, M	1	Aligarh
<i>Vernonia cinerea</i> (L.) Less.	S	1	"

\* New host record.

\*\* First report from India.

Dept. of Botany.

S. QAMAR A. NAQVI.  
Aligarh Muslim University, M. MASHKOOB ALAM.  
Aligarh, India, June 8, 1974.

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***Geocoris jucundus* Fieb. \* (Lygaeidae : Hemiptera) as Predator of Lucerne and Tomato Aphids in the Indian Desert**

Species of *Geocoris* are usually predatory in habit. *G. tricolor*, found in India, feeds on nymphs and adults of brinjal tingid bug, *Urentius sentis* and mite, *Tetranychus* sp., sorghum shoot bug, *Peregrinus maidis* (Rawat and Modi, 1969)<sup>1</sup>, adults of coccinellid, *Brumus saturnalidis* and anthorid bug, *Orius* sp. (Singh and Sandhu, 1973)<sup>2</sup>.

During February and July 1970 the author collected at the Central Research Farm, C.A.Z.R.L. Jodhpur, *Geocoris jucundus* Fieb. feeding on lucerne aphid, *Therioaphis trifolii*, which is a common and widespread pest of lucerne. This predator has also been recorded on tomato aphid, *Aphis gossypii*, at C.R. Farm, Jodhpur. *G. jucundus* is being reported for the first time from the Indian Desert.

Central Arid Zone

S. K. PAL.

Research Institute,  
Jodhpur, May 29, 1974.

\* Identified by Commonwealth Institute of Entomology, London SW 7.

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## REVIEWS AND NOTICES OF BOOKS

**Calculus of Several Variables.** By Serge Lang. (Addison-Wesley Pub. Co., Inc., Reading, Massachusetts 01867, U.S.A.), 1973. Pp. viii + 376. Price not given.

This book is a welcome arrival. It is nice to devote a separate book for calculus of several variables, because it is likely that this topic gets comparatively less space and hence cramped treatment when it is included in the same book in which calculus of one variable is presented.

This book is organised in four parts. Part one has 7 chapters and is devoted to mappings from numbers to vectors and vectors to numbers. It introduces the reader to vector preliminaries, differentiation of vectors, chain rule, gradient, potential function, curve integrals, higher derivatives, and maxima and minima including the Lagrange multipliers. Part two has 3 chapters, one each for matrices, linear maps and determinants. Part three has only one chapter that considers mappings from vectors to vectors discussing applications to functions of several variables. Multiple integrals, change of variables, Green's theorem, and surface integrals are studied in 4 chapters of the fourth part. This organisation helps the author to go little deeper, if necessary, in one part than is required, to read another part and similarly helps the reader to omit certain in-between portions without impairing his understanding of later portions. For example, one can go to study the 12th chapter on multiple integrals immediately after the first chapter on vector algebra. The book has one appendix on Fourier series. Every section of each chapter has exercises and answers to selected exercises are included.

Study of vectors is basic to that of functions of several variables. The author has to be selective in the treatment of vector analysis as it is to be used only as a tool for the study of main concern of the book. As such scalar and vector triple products, reciprocal system of vectors do not find place in this book. The treatment of vectors is not geometry-dominant but lays algebraic foundations. Scalar product of two vectors  $A$  and  $B$  is defined as  $\sum a_i b_i$  and not as  $|A| \cdot |B| \cdot \cos \theta$ . The author has pointed out the advantages of this approach. The author is careful in stating that the section on the cross product applies only in 3-space. The cross product introduced in the last section of first chapter is used only in the last chapter, viz., 15th on

surface integrals. In the rules for the derivatives, derivative of dot product is given but not of cross product. The traditional unit vector notation  $i, j, k$  do not find place in the book.

The treatment of topics in the second part is kept to the bare minimum. For example, only second and third order determinants are introduced. Part three is an illustration of the fact that "analysis profits from algebra, and conversely, the algebra of linear mappings finds a neat application which enhances its attractiveness". The chain rule for arbitrary compositions of mappings is established. Further the important Inverse mapping theorem, and Implicit function theorem are included. The treatment of part four is well structured.

Figures in the book are attractive. Printing and set-up is good. Printer's devil like 'Exercise 5' instead of 'Exercise 6' in the 7th line from bottom on page 21 is rare. The whole book is neatly and very carefully planned and written and is really a good text-book.

V. G. TIKEKAR.

**Mössbauer Effect and its Applications.** By V. G. Bhide. (Tata McGraw-Hill Publishing Co., Ltd., New Delhi), 1973. Pp. 500. Price Rs. 75-00.

Mössbauer spectroscopy has established itself as a research tool and as an analytical method, and a book covering all aspects of the subject is most welcome. Dr. Bhide, who is currently the Deputy Director of the National Physical Laboratory, New Delhi, pioneered the work in the field in our country, soon after the discovery of recoil less resonant absorption of gamma-rays by Mössbauer, and has made substantial contributions to various aspects of Mössbauer studies, e.g., in ferroelectrics, ferrites and magnetic materials, chemical binding, etc.

This book strikes a healthy balance between the different aspects of the subject—theory, instrumentation, current status of the applications to chemical binding, magnetism, ferroelectricity and potential applications to different areas, including biosciences. A brief introduction and treatment of the theory of the effect is followed by a description of the instrumentation involved. Applications to lattice dynamics and studies of atomic motion in solids and liquids are followed by a brief review of ferroelectricity and applications of Mössbauer effect of ferroelectric studies.

The chapter on applications to magnetism gives a succinct account of the origin of the hyperfine field and magnetic hyperfine structure in Mössbauer spectra followed by the fairly exhaustive review of the applications to alloys, ferrites, garnets, etc., and an inter-comparison of the results with those obtained by other techniques for investigation of hyperfine interactions such as EPR, NMR and perturbed angular correlation studies. This is followed by a chapter on the applications to chemical binding where isomer shifts and quadrupole splitting effects are elucidated and their application to Fe and Sn compounds in various oxidation states reviewed, as also the relevant calculations, and an inter-comparison with other techniques as X-ray spectroscopy, and electron spectroscopy which have a bearing on the nature of the chemical bond. The last chapter gives a brief review of the applications to biosciences which are currently attracting considerable interest.

This is a well-written book which can serve both as a text and a source-book, especially to graduate students intending to work in this field whose special attraction lies in the range and versatility of problems that could be studied, and the not-so-expensive nature of the instrumentation involved. A cheaper paper back edition of the book would certainly prove useful.

V. S. VENKATASUBRAMANIAN.

## ANNOUNCEMENTS

### Conference to Take Stock of Water Problems

Experts from approximately 100 countries will meet at Unesco from September 2 to September 14 to take stock of water resources throughout the world and adopt plans for further co-operation.

The international conference is being organized by Unesco and the World Meteorological Organization (WMO) to review the achievements of the International Hydrological Decade and discuss the draft plan of the International Hydrological Pro-

gramme to be presented to the General Conference of Unesco in October. At the same time, Unesco headquarters will be the scene of celebrations of the 300th anniversary of scientific hydrology.

As part of the Decade, Unesco has promoted research on the world water balance, the study of floods and low flow, experimental and representative basins, the study of man's influence on the water cycle and groundwater hydrology. In addition to these international efforts, countries with similar climatic and water conditions have undertaken joint studies such as in Scandinavia, the Danube and Baltic countries.

### Transducer Symposium

An All India Symposium on Transducer Technology will be held at COCHIN, Kerala, on the 15th and 16th of October 1974.

Dr. B. D. Nag Chaudhuri, Vice-Chancellor of Jawaharlal Nehru University, will inaugurate the Symposium and Prof. M. G. K. Menon, Scientific Adviser to the Ministry of Defence, will preside on the Symposium.

Over 120 organizations will participate at the Symposium at which 70 papers will be read.

The two-day Symposium will also feature an exhibition of commercial and scientific transducer products.

The further details please contact the Convener Shri C. Madhavan, Assistant Director of the Naval Physical and Oceanographic Laboratory, Naval Base, Cochin-4.

### Books Received

*Quantum Mechanics in a New Key.* By Alfred Lande (Exposition Press, Inc., 50, Jericho turnpike, Jericho, New York 11753), 1973. Pp. x + 131. Price \$6.50.

*Teasing Relationship.* By Richard W. Howell. (Addison-Wesley Publishing Co., Inc., Reading, Massachusetts, 01867), 1973. Pp. 22. Price not given.

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# SCINTILLATIONS OF SATELLITE SIGNALS NEAR MAGNETIC EQUATOR

H. CHANDRA AND R. G. RASTOGI

Physical Research Laboratory, Ahmedabad 380009 (India)

## ABSTRACT

The nocturnal, seasonal and magnetic activity variations of scintillation of radio beacon signals at equatorial regions are shown to be very closely associated with similar changes of spread-F. The belt of high scintillation is about 1000 km wide and is centred within about 5° from the dip equator. The same irregularities are suggested to cause spread-F and radio scintillations at low latitudes.

## INTRODUCTION

THE present paper describes the results of a study of scintillation of radio beacon signals on 40.010 MHz radiated from Explorer 22 and Explorer 27 satellites as recorded at Thumba (0.6° S dip).

The scintillation index used in the present study is determined as described by Aarons *et al.* (1963). The maximum ( $I_x$ ) and minimum ( $I_n$ ) levels of scintillation were determined and index calculated as

$$SI = \frac{(I_x - I_n)}{I_x + I_n}$$

Normally the calculations are made for the sub-ionospheric latitude of 8° N which corresponds to ionosphere over Thumba. For the latitudinal variation of scintillation index, it is calculated at every 15 second interval. Apart from the calculations of scintillation index, fading rate was also determined for the sub-ionospheric latitude of Thumba.

## DAILY AND SEASONAL VARIATIONS OF THE SCINTILLATION INDEX

The following parameters of the scintillations of the radio waves were computed for the latitudes of Thumba: (1) percentage occurrence of the scintillations, (2) scintillation index and (3) fading rate of the signals. Figure 1 shows the annual mean nocturnal variations of these parameters which are compared with the mean nocturnal variation of the spread-F index at Thumba. A remarkable similarity is seen between all the four curves, indicating that the various parameters of the scintillation of radio waves at Thumba are very closely associated with the spread-F. Both the spread-F and radio wave scintillations have peak around 20–22 hr and around 03–04 hr.

The spread-F at Thumba is basically of two types: (1) Range spread which occurs mostly around 22 hr and is more frequent during equinoxes than during solstices. (2) Frequency spread which occurs mostly during pre-sunrise hour and seasonally during summer-J months (Chandra and Rastogi, 1972, 1973). The scintillation indices were computed separately for 20–23 hr and 02–05 hr of each season. The corresponding spread-F indices

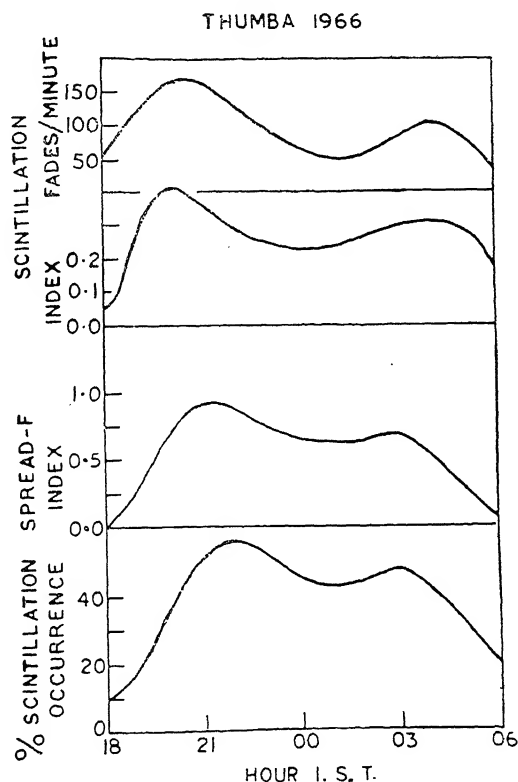


FIG. 1. Annual average nocturnal variations of scintillation occurrence, spread-F index, scintillation index and fading rate of scintillations at Thumba during the year 1966.

were also computed and both the indices are collected in Table I.

TABLE I  
Mean scintillation index and spread-F index for different seasons during the year 1966

Parameter	20–23 hr			02–05 hr		
	Months			Months		
	D	E	J	D	E	J
Scintillation index	0.28	0.33	0.17	0.10	0.05	0.50
Spread-F index	0.66	1.30	0.45	0.38	0.50	0.70

For the pre-midnight period 20–23 hr both the scintillation as well as the spread-F indices are highest during equinoxes and lowest during J months. For the pre-sunrise period both the indices are again highest during J months. Thus the scintillation at an equatorial station is found to be caused by both the types of spread-F.

#### MAGNETIC ACTIVITY AND SCINTILLATION

To study the effect of magnetic activity on scintillation at Thumba,  $\Sigma Kp$  of each day was classified in one of the three groupings, i.e.,  $\Sigma Kp$  between 0–10, 11–20 and 21–30. Average scintillation index for the three groups were computed and are given in Table II. The scintillation index drops from

TABLE II

Mean scintillation index for days with different  $Kp$

$Kp$	No. of observations	Mean scintillation index
0–10	44	0.34
11–20	47	0.23
21–30	11	0.17

0.34 ( $\Sigma Kp$  between 0–10) to 0.23 ( $\Sigma Kp$  between 11–20) and to 0.17 ( $\Sigma Kp$  between 21–30). Thus at Thumba the scintillation activity is systematically reduced with increasing magnetic activity. The spread-F index at Thumba (Chandra and Rastogi, 1972) is already shown to decrease with magnetic activity.

For African zone both the spread-F index (Halley and Gatty, 1966) and the scintillation index (Koster and Wright, 1963; Koster, 1972) are found to decrease with magnetic activity. Bandyopadhyay and Aarons (1970) did not find any clear relationship between the scintillation and magnetic activity at Huancayo.

Later on Mullen (1973) found that the scintillation at Huancayo tends to increase with magnetic activity during June solstices. Further, whole night average index for December solstice shows no change with magnetic activity. For equinoxes the magnetic disturbance tends to decrease the scintillation for the pre-night hours and tends to increase the scintillation for the post-midnight hours. It is to be noted that there is a remarkable similarity between these results and the results by Chandra and Rastogi (1973) for the effect of magnetic activity in the spread-F at Huancayo. Thus the correlation between the scintillation and spread-F is very high for equatorial stations.

#### LATITUDINAL VARIATION OF SCINTILLATION INDEX NEAR THE MAGNETIC EQUATOR

Some N–S passes were studied in detail for obtaining the latitudinal profile of scintillation index. Figure 2 shows the variation of scintillation index for few passes. It is seen that the belt of high scintillation index is of the order of 8–10 degrees which corresponds to roughly 1000 km. This belt is not always centred on the dip equator and can be displaced by about 5 degrees north or south of the dip equator.

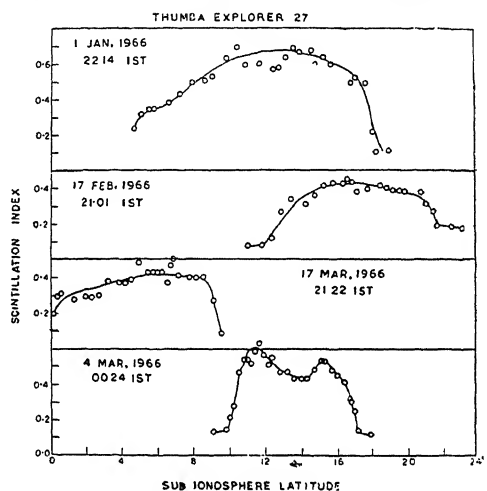


FIG. 2. Scintillation index vs sub-ionospheric latitude plot for a few explorer 27 passes during the year 1966.

#### ACKNOWLEDGEMENT

Grateful thanks are due to late Prof. V. A. Sarabhai, former Chairman of Indian National Committee for Space Research, for the encouragements and facilities provided towards the establishment of the ionospheric research station in Thumba. Thanks are also due to our colleagues Dr. S. Ramakrishna, Mr. T. Vergese and Mr. K. Sen Gupta for their co-operation during the course of work.

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## COSTUS SPECIOSUS RHIZOME AS SOURCE OF DIOSGENIN

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### ABSTRACT

Rhizomes of *Costus speciosus* Sm., a perennial herb having a wide distribution in India, have been found to contain upto 2.6% of diosgenin. The plant can be easily propagated through rhizome cuttings and have good prospects of becoming a raw material for steroid industry in India.

### INTRODUCTION

**D**IOSGENIN is one of the sapogenins extensively used in the manufacture of steroidal hormones. The raw material for it consists mainly of the underground parts of a few species of *Dioscorea*, occurring wild in Mexico, South America, India and China. The demand for steroidal hormones, especially those used as antifertility drugs has increased in recent years and the fears are expressed about an early exhaustion of the raw materials due to difficulties in cultivating high yielding varieties of *Dioscorea*. A search for substitute raw materials is being carried out in different parts of the world but none of the new plants found to contain diosgenin have provided enough evidence of becoming a commercial success. In India, rhizomes of *Dioscorea deltoidea* Wall. and *Dioscorea prazeri* Prain and Burkill, occurring wild in Himalayas (diosgenin content of 3.5% and 2.0% respectively)<sup>3</sup> are the only raw materials utilized by steroid industry. The supplies available from these two sources are however likely to exhaust in the next ten to fifteen years due to large collections from the forest areas, poor natural regeneration and failure in raising these plants as commercial crops. A number of other plants, viz., *Trillium govanianum*, *Balanites roxburghii* and *Paripolyphylla* have been reported to contain diosgenin<sup>2,4,5</sup> but there is hardly any scope for their commercial exploitation due to a very low diosgenin content or poor availability. Recently Das Gupta and Pandey<sup>1</sup> reported the occurrence of diosgenin in the rhizomes of *Costus speciosus* Sm., a perennial herb commonly available in different parts of India. This report led the authors to undertake detailed investigations on utilization of the plant for commercial production of diosgenin.

### BOTANICAL CHARACTERISTICS

*Costus speciosus* Sm. (Fam.: Zingiberaceae) is an erect herbaceous plant upto 2 m high with long lanceolate leaves and white fragrant flowers in terminal clusters. The plant flowers during the months of July and August, the aerial parts withering away during winter months. It has a very wide distribution in India, occurring throughout the sub-Himalayan tract from Himachal Pradesh to Assam, Vindhya and Satpura hills in Central India and the western ghats of Maharashtra, Karnataka

and Kerala. It is more commonly found in the moist shady localities under the mixed deciduous forests upto an elevation of 1200 m above mean sea level.

### DIOSGENIN CONTENT OF THE RHIZOME

Detailed chemical examinations of the rhizome was done for this purpose. In the first experiment 250 g of dried rhizomes were hydrolysed with 10% hydrochloric acid, filtered, washed and exhausted with *n*-hexane. The extract on concentration gave 5 g (2%) of diosgenin needles (201° C–203° C). These when crystallised from ethyl alcohol yielded fine needles (205° C–206° C) showing no depression in melting point when mixed with authentic samples of diosgenin. Further the benzene and chloroform extracts yielded nothing whereas the alcoholic extract designated as fraction A showed one major spot moving with the solvent front on T L C (Silica gel; hexane : ethyl acetate :: 80 : 20). This fraction was further subjected to resolution through different solvent systems (benzene *R<sub>f</sub>* 0.25; *n*-hexane : ethyl acetate 95 : 5, *R<sub>f</sub>* 0.47; petroleum ether : 90 : 10 *R<sub>f</sub>* 0.53), but it always showed the major spot to be a single entity. In another experiment, 300 g of dried tubers were defatted with petroleum ether and then exhausted with 85% alcohol. The alcoholic extract was concentrated to remove as much alcohol as possible and the residue hydrolysed and processed as usual. This yielded about 7.5 g (2.5%) of a brownish solid designated as fraction B which (*n*-hexane : ethyl acetate; 80–20): revealed four spots on T L C, one corresponding to authentic diosgenin (*R<sub>f</sub>* 0.51), another moving with the front (*R<sub>f</sub>* 0.95) and the rest two minor spots with *R<sub>f</sub>* values as 0.56 and 0.87. It was then chromatographed over alumina (110 g) when *n*-hexane fraction gave a mixture of the *R<sub>f</sub>* 0.87 and 0.95 spots, the latter very much predominating (fraction C). The spot with *R<sub>f</sub>* 0.95 when run concurrently with fraction A was found to be identical with major spot in the latter and moved parallel in all the solvent systems studied above. Subsequent elution of the column with 10% benzene in petroleum ether gave predominantly diosgenin (1.95%) admixed with *R<sub>f</sub>* 0.56 spot (fraction D). This last fraction on single crystallization from ethyl alcohol gave white needles



(m.p. 204° C–205° C) which when mixed with authentic diosgenin showed no depression. As fractions A and C were similar in T L C pattern, they were combined and rechromatographed over alumina. The *n*-hexane fractions yielded a white crystalline material which on a single crystallization from ethyl alcohol gave white needles positive to Liebermann Burchard test for sterols.

Das Gupta and Pandey<sup>1</sup> obtained 3.88% of an extractive (ethyl alcohol) which they designated as sapogenins. This further yielded 2.12% of pure diosgenin. In the present investigation the percentage of pure diosgenin (m.p. 204° C–205° C) obtained is about 2% out of a total extractive of 2.5%. The above workers did not investigate the nondiosgenin part which could have been sapogenins or a mixture of sapogenins and some other compounds because the rhizomes were extracted with ethyl alcohol. Further elucidation work on this fraction is in progress. It was further observed that the quality of diosgenin obtained from *Costus speciosus* is better than that obtained from *Dioscorea deltoidea* (whiter colour and a melting point of 201° C–203° C against 190° C of diosgenin obtained from the latter). Comparative T L C of the diosgenin obtained from the two plants confirmed the above observations.

#### RAW MATERIAL RESOURCES

Reconnaissance carried out in various parts of India revealed a fair availability of *Costus* rhizomes in the sub-Himalayan tract of Himachal Pradesh, Uttar Pradesh and Nepal in the north and the Ghat areas of Maharashtra, Goa, Karnataka and Kerala in western and southern India. A lot of variation in diosgenin content ranging from 0.38% to 2.6% has been noticed in rhizome samples collected from various localities. The samples obtained from the sub-Himalayan tract have higher diosgenin content than those from western and southern India. A further investigation on the diosgenin variability was carried out. Rhizome samples from 20 plants growing side by side were collected from 2 different localities (Kangra in Himachal Pradesh and Dehra Dun in Uttar Pradesh) and chemically analysed. The diosgenin content was found to vary from plant to plant ranging from 0.58% to 2.63%.

Possibilities for cultivating high yielding strains (diosgenin content 2% or more) were therefore examined. Rhizomes of the plant were cut in small pieces (each weighing about 35 g) and were put in well-prepared soil in rows at a distance of 0.5 m from plant to plant as well as from row to row during the month of March. These pieces sprouted in the last week of May. The plants attained a height of about 1 m towards the end of July when profuse flowering also ensued. The growth of plant was comparatively better in well-irrigated plots. The aerial portions of the plant

withered away in December and rhizomes remained dormant till next May. To check the increase in weight in the underground parts the rhizomes were dug up in the month of January, i.e., after 9 months of planting. These had attained a good size and weighed 1570 g on an average. The increase in size from 35 g (seed tuber) to 1570 g (maximum harvest) was very encouraging. The rhizomes on chemical analysis, however, showed a decline in diosgenin content from 2.3% to (average) 1.6%. Experiments are in progress to determine the correlation between the age of the plant and the diosgenin content.

#### CONCLUSIONS

The present investigations reveal great potentialities for utilization of *Costus speciosus* rhizomes as a substitute raw material for the production of diosgenin in India. The yield of diosgenin is comparatively low, being 1.5% to 2% against 3% to 4% in the rhizomes of *Dioscorea deltoidea* and 2% in the rhizomes of *Dioscorea prazeri*. There are, however, a number of points which well compensate the low yield of diosgenin. These are

1. The plant has a very wide distribution zone extending from sub-Himalayan tract in the north to hilly regions of Central India and the western ghats, and can be collected in appreciably large quantities.

2. The diosgenin obtained from it is far more pure than that obtained from *Dioscorea* spp.

3. The plant can be easily propagated even in the plains. Preliminary cultural trials show a very fast growth of underground portions with a slight decline in diosgenin content.

As stated above, the supplies of *Dioscorea* rhizomes are fast depleting due to large scale extraction from the forest areas. The situation is further aggravated due to failures in raising Indian *Dioscoreas* as commercial crops. In the light of these facts the development of *Costus speciosus* Sm. as a raw material for steroid industry becomes more important. Attempts towards large scale introduction of clones with a diosgenin content of 2% or above are being made together with technoeconomic studies for commercial utilization of this new raw material. The findings will form the subject-matter of a further communication. The authors thank Shri Vir Singh and Shri J. P. Singh for assistance rendered in the work.

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# IDENTITY AND NOMENCLATURE OF *UTRICULARIA NIVEA* VAHL

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*Botanical Survey of India, Calcutta*

## ABSTRACT

The paper deals with the identity and nomenclature of *Utricularia nivea* Vahl, and *Utricularia caerulea* Linn. Detailed descriptions of these two species and the features on which they can be identified are given.

WHILE studying the various species of *Utricularia* occurring in India, some discrepancy regarding the identity of *Utricularia nivea* was noted, which is presented here.

C. B. Clarke in J. D. Hooker's *Fl. Brit. India* 4 : 333, 1884, cites *U. nivea* Vahl as a synonym to *U. racemosa* var. *flicaulis* (Wall. ex DC.) Clarke. Gamble in *Fl. Pres. Madras* 2 : 691, 1957 (repr. edit.) has shown that *U. racemosa* Wall. ex DC. is synonymous with *U. caerulea* Linn. non sensu Clarke in *Fl. Brit. India* 4 : 331, 1884 nec Cooke in *Fl. Pres. Bombay* 2 : 392, 1958 (repr. edit.) quae est *U. graminifolia* Vahl. Cooke loc. cit. p. 393 treats *U. nivea* Vahl, *U. flicaulis* Wall. ex DC. and *U. racemosa* Wall. ex DC. as conspecific

*flicaulis*, Clarke ... the flowers are sparse and distant".

A careful examination of all the herbarium sheets available in the various herbaria of India and the preserved materials available to the authors revealed that *U. nivea* is quite distinct from *U. caerulea*. As for the colour variation of the flowers pointed out by Merrill, there is a herbarium sheet, *Vicary s.n.* (CAL) with the following remarks "... in no way differing except for colour which is purple ...". The study of the seeds from the above sheet further corroborates Merrill's findings of colour variation in *U. nivea* Vahl.

These two species can be distinguished in the following features :

### *U. nivea* Vahl

1. Flowers white (rarely purple), sparse and not crowded towards the apex of scape.
2. Corolla—upper lip shallow to deeply bifid at apex; lower lip less than half the length of spur.
3. Seeds with clavate projections.

and selected the earliest valid binomial of the three, *U. nivea* Vahl. It was Merrill in *Philipp. J. Sci.* 7 : 347, 1912, who for the first time recognised *U. nivea* as distinct from *U. caerulea* and remarked that "The Philippine plants however have pale purplish flowers rather than white as in the type of Vahl's species".

Gamble loc. cit. and Santapau in *J. Bombay nat. Hist. Soc.* 49 : 220, 1952, treat *U. racemosa* Wall. ex DC. and *U. nivea* Vahl as synonymous with *U. caerulea* Linn. Haines also in *Bot. Bihar and Orissa* 2 : 676, 1961 (repr. edit.) treats *U. racemosa* Wall. ex DC. and *U. flicaulis* Wall. ex DC. as synonymous to *U. caerulea* Linn. Clarke differentiated the variety *flicaulis* from *U. racemosa* and stated "stem slender with fewer scattered flowers". Gamble casually mentions, "The very small form with few usually white flowers is var. *flicaulis*, C. B. Clarke". Haines also mentions, "... in var.

### *U. caerulea* Linn.

- Flowers blue or purplish, mostly crowded towards the apex of scape.
- Corolla—upper lip truncate to emarginate at apex; lower lip more than half the length of spur.
- Seeds without clavate projections.

The features of the seeds are shown in Figs. 1 and 2. Hence it is necessary to segregate *U. nivea* Vahl from *U. caerulea* Linn. and treat the former as a distinct species. The nomenclature and descriptions of the two species are given below :

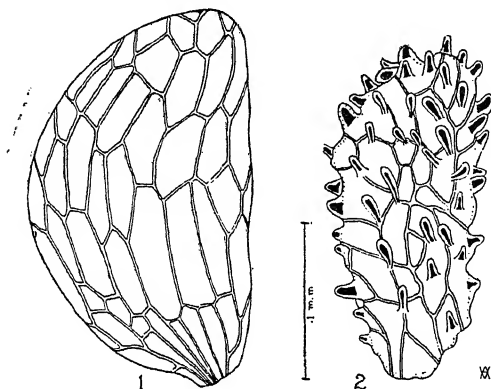
*U. nivea* Vahl, *Enum.*, 1 : 203, 1805; *Wt. Icon. t.* 1582, 1850; Merrill in *Philipp. J. Sci.* 7 : 247, 1912. *U. flicaulis* Wall. (Cat. 1501, 1829, nom. nud.) ex DC. *Prodr.* 8 : 21, 1844. *U. racemosa* var. *flicaulis* (Wall. ex DC.) Clarke in *Hook. f. Fl. Brit. India* 4 : 333, 1884.

Terrestrial herbs; roots few, simple, capillary. Leaves up to 5 mm long, linear-spathulate, 1-nerved, rounded at apex. Scape erect, 4.5–15.5 cm tall, slender, glabrous; scales few, attached by the middle, up to 2 mm long, 1-nerved, acute at apex. Flowers usually few, sparsely arranged; pedicels about 1 mm long; bracts medifixed, up to 2 mm long, 1-nerved, acute at apex; bracteoles attached apparently by base, up to 1 mm long, 1-nerved, acute at apex; calyx lobes up to 1.5 mm long,

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<sup>2</sup> Tata Silk Farm, Bangalore-4.

minutely papillose, 5-6-nerved, rounded at apex; corolla white (rarely purple); upper lip 2 mm long, oblong to slightly expanded above, shallow to deeply bifid at apex; lower lip ovate, bent down covering less than half the length of spur; spur conical, minutely papillose; ovary ovoid, up to 1.5 mm long. Capsules ovoid, up to 2 mm long; seeds ovoid, reticulate with clavate projections.



FIGS. 1-2. Seeds of two species of *Utricularia*; Fig. 1. *U. caerulea*. Fig. 2. *U. nivea*.

*Specimens examined*:

INDIA. ASSAM: Khasia mountains, *Hooker f. et Thompson s.n.* (CAL). BENGAL: Sili-guri, *King s.n.* (CAL); Titalyah, *Kurz s.n.* (CAL). MADHYA PRADESH: Guna, *Ridley 28* (CAL). MYSORE: Yellapur, North Canara, *Talbot 1047* (CAL). ANDHRA PRADESH: Mahimandalam, Chittoor District, *Fischer 4685* (CAL); Madanapally, Chittoor District, *Fischer 4712* (CAL). MADRAS STATE: Annamallay, *Wight 2418* (CAL). KERALA: Munnar, *Saldanha 8045* (BLAT).

BURMA. Tavoy, *Wallich 1501* (CAL); Kaukkwe valley, Bhamo District, *Lace 6056* (CAL).

MALACCA. *Ridley 10706* (CAL).

NO LOCALITY. *Vicary s.n.* (CAL).

*U. caerulea* Linn. *Sp. Pl.*, 1: 18, 1753; *Wt. Icon.* t. 1583, 1850; *Gamble, Fl. Pres. Madras* 2: 691, 1957 (repr. edit.), excl. syn. *U. nivea* Vahl; *Haines, Bot. Bihar and Orissa* 2: 676, 1961 (repr. edit.), p.p.; *Santapau in J. Bombay nat. Hist. Soc.* 49: 220, 1952, excl. syn. *U. nivea* Vahl. *U. racemosa* Wall. (Cat., 1496, 1829, nom. nud.), ex DC. *Prodr.* 8: 21, 1844; *Clarke in Hook. f. Fl. Brit. India* 4: 333, 1884; *Prain, Bengal Pl.* 2: 582, 1963 (repr. edit.). *U. nivea sensu Cooke, Fl. Pres. Bombay* 2: 393, 1958 (repr. edit.).

Terrestrial herbs, roots many, capillary, simple stolons numerous from the base of scape, branched.

Traps ovoid, dimorphic; mouth terminal with an oblique funnel-shaped rim, margin of rim covered with glandular hairs; rim of the upper lip produced into a long carinate beak. Leaves few, up to 1.9 cm long, linear-spathulate, 1-nerved, rounded at apex. Scape erect, 3.5-30 cm tall, stout, glabrous; scales few, attached by middle, up to 6 mm long, 1-nerved, acute at apex. Flowers usually many in congested racemes; pedicels up to 1 mm long; bracts medifixed, up to 5 mm long, 1-nerved, acute at apex; bracteoles attached above base, 1-nerved, up to 4 mm long, acute at apex; calyx lobes up to 5 mm long, minutely papillose, 7-9-nerved, rounded at apex; corolla blue or purple; upper lip 4-6 mm long, base ovate-deltoid, limb obovate to suborbicular, truncate or emarginate at apex; lower lip entire, bent down covering more than half the length of spur; spur conical, 7-8 mm long, curved forward or sometimes upwards, tapering and acute at apex, minutely papillose; ovary ovoid, 2-3 mm long. Capsules ovoid, up to 3 mm long; seeds obovoid, smooth-walled, reticulate.

*Specimens examined*:

INDIA. ASSAM: Khasia, *Clarke 15164* (CAL); Mamloo, Khasia, *Clarke 45291* (CAL); K. and J. Hills, *Deka 18537* (ASSAM) K. and J. Hills, *Deka 22985* (ASSAM). BENGAL: Silhet, *Wallich 1496/1* (CAL) Dinajpur *Vicary s.n.* (CAL); Manbhum, *Campbell s.n.* (CAL). BIHAR: Chota Nagpur, *Prain s.n.* (CAL); Hazaribagh, Chota Nagpur, *Clarke 850* (CAL); Singhbhum, *Haines 208* (CAL); Mihijam, Santhal Parganas, *N. Gill s.n.* (CAL). ORISSA: Ramagiri, *Subba Rao 30354* (ASSAM). MADHYA PRADESH: Satna town, *Sebastine 8899* (MH); Rajbandha tank, Bastar District, *Subramanyam 7162* (MH). MAHARASHTRA: Amaravathi road, Nagpur, *Nafday 59* (BSI); Ambazeri tank, Nagpur, *Subramanyam 4683* (MH). MYSORE: North Canara, *Talbot 1047B* (BSI). ANDHRA PRADESH: Madanapally, Chittoor District, *Fischer 4710* (CAL). MADRAS STATE: Shonthethy canal, Kodaikanal, *Barber 7531* (MH); Ramnad District, *Ramamurthy 22732, 22733* (MH). KERALA: Kamalakad, Wynad, *Saldanha 7513* (BLAT).

BURMA. Pegu, *Kurz 2306* (CAL); *Griffith 4080* (CAL).

MALAYA. Larut, Perak, *King 1931* (CAL); Perak, *Kunstler 3820* (CAL); Taiping, Perak, *Wray 131* (CAL); Penang, *Curtis s.n.* (CAL).

HONGKONG. Fokien, *Dunn 3366* (CAL).

CEYLON. *Thwaites 277* (CAL).

# CYTOPHOTOMETRIC ANALYSIS OF ASCORBIC ACID (AA), RIBONUCLEIC ACIDS AND SULFHYDRAL PROTEINS DURING EMBRYO GENESIS IN *COIX LACRYMA JOBI* L.

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## ABSTRACT

Histochemical studies reveal that all the organogenetic centres of the proembryo are uniformly stained. At first indentation, a difference in localisation pattern arises. The globular embryo is physiologically the most active stage in development. Positive correlation is shown to exist by the regressions.

**F**RESH fertilised carpels of *Coix* were fixed in the fluids of Maheshwari<sup>1</sup>, Carnoy<sup>2</sup>, and neutral formalin. The sections were stained with pyronin<sup>2</sup> G for RNA and DDD test of diazo red<sup>3</sup> for -SH. Alcoholic silver nitrate at 0-3° C and pH 2-2.5 was used as a fixative for the localisation<sup>4</sup> of ascorbic acid due to its quick penetration and the specificity<sup>5</sup> of the reaction. Control slides were prepared for all the metabolites. The absorbance of the exposed chromophore was measured by a simple cytophotometer<sup>6</sup> devised in our laboratory. The assembly follows Beer's law of linear relationship between the absorbance and section thickness. The green filter (500-570  $\mu$ m) served for red chromophore of RNA and -SH while the measurement of black silver grains was performed under

the concentration per unit area of the cell. Correlations between AA and RNA, AA and -SH and RNA and -SH were established by the regression method. E. values were used to calculate the regression equations and to trace their trend lines.

The zygote is a strongly polarised cell. Its proximal pole shows vacuolated cytoplasm containing less amount of AA, RNA and -SH proteins while the distal pole shows a strong staining reaction. As the development proceeds, the stain intensity (e. values) for AA, RNA and -SH in the derivatives of *ca* rises and reaches its peak when the embryo is globular. Another small peak is visible when the second indentation takes place. At maturity the stain intensity considerably declines. The amount of these metabolites (Table I)

TABLE I

*The quantitative data of ascorbic acid, RNA and -SH proteins during embryogenesis*

Stage of embryogenesis	Extinction value (e. value)			Cell area (in $\mu^2$ )	Content per unit area of the tissue		
	Ascorbic acid	RNA	-SH Protein		Ascorbic acid	RNA	-SH Protein
Zygote	.. 0.13	0.06	0.04	5396.6	701.6	323.8	215.9
Pro-embryo—							
(a) 2-celled	.. 0.21	0.13	0.08	2,860.0	600.6	114.4*	288.8
(b) 4-celled	.. 0.22	0.22	0.17*	2,376.3	522.8	522.8	403.98*
(c) 16-celled	.. 0.33	0.23	0.19	1,577.9	520.4	351.9	300.7
Globular embryo	.. 0.63	0.47	0.28	1,731.5	1,107.2	815.5	326.7
Indented embryo—							
(a) First indentation	.. 0.44	0.24	0.1	1,251.1	564.5	310.3	129.5
(b) Second indentation	.. 0.37	0.3	0.27	1,464.3	669.2	468.0	467.7
Mature embryo	.. 0.32	0.05	0.13	1,633.5	492.0	90.7	188.0

\* Values derived from the regression equation.

white light. E. values were multiplied by cell area to obtain the total content of the metabolite per cell. E. values are divided by cell area to obtain

(expressed in terms of content per unit area) shows similar changes observed for the stain intensity except one fall at the 16-celled pro-embryo. This

TABLE II

The regression values of ascorbic acid, RNA and -SH proteins in different combinations

Stage	Regression values of ascorbic acid (X) <i>versus</i> RNA (Y)				Regression value of ascorbic acid (X) <i>versus</i> -SH protein (Y)				Regression value of RNA (X) <i>versus</i> -SH protein (Y)				
	$X = 0.71 Y + 0.1838$ $Y = 0.8 X - 0.0042$				$X = 1.4 Y + 0.12$ $Y = 0.51 X - 0.05976$				$X = 0.02 + 1.25 Y$ $Y = 0.07 + 0.47 X$				
	X	XI	Y	YI	X	XI	Y	YI	X	XI	Y	YI	
Zygote	..	0.13	0.22	0.06	0.06	0.13	0.176	0.04	0.06	0.06	0.07	0.04	0.0982
Pro-embryo—													
(a) 2-celled	..	0.21	..	..	0.126	0.21	0.332	0.08	0.04	0.13	0.04	0.08	..
(b) 4-celled	..	0.22	0.34	0.22	0.13	0.22	..	0.17	0.052	0.22	..	0.17	0.1734
(c) 16-celled	..	0.33	0.35	0.23	0.22	0.33	0.38	0.19	0.108	0.23	0.2575	0.19	0.1838
Globular embryo	..	0.63	0.51	0.47	0.46	0.63	0.51	0.28	0.26	0.46	0.37	0.28	0.2905
Indented embryo—													
(a) First inden- tation	..	0.44	0.36	0.24	0.31	0.44	0.26	0.1	0.164	0.24	0.145	0.1	0.1838
(b) Second inden- tation	..	0.37	0.39	0.3	0.25	0.37	0.498	0.27	0.128	0.3	0.3575	0.27	0.211
Mature embryo	..	0.32	0.22	0.05	0.21	0.32	0.302	0.13	0.103	0.05	0.1825	0.13	0.0932

is related to the small size of the embryonal cells at this stage. The globular embryo has the highest rate of synthesis of AA, RNA and -SH proteins (Fig. 1). Its physiological state is most active. The tiered arrangement of four, eight and sixteen cells does not show any difference in the staining reaction. The organogenetic centres of the pro-embryo are all physiologically equally active. Schultz and Jensen<sup>7</sup> could not detect any apparent differences until the formation of a heart-shaped embryo in *Capsella*. The first difference in the localisation pattern in *Coix* is observed during the first notch in the elongated embryo. At this stage the germinal face which is the future plumule-radical axis reveals a higher staining reaction than

the abgerminal face which forms the scutellum. At maturity the staining intensity decreases indicating the slowing down of the synthetic processes.

In the regression of these metabolites (Table II), the points representing the e. values of AA, RNA

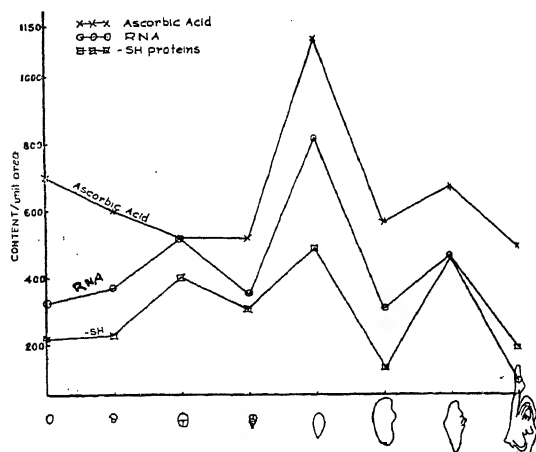


FIG. 1. Relative changes in the content of AA, RNA and sulfhydryl proteins per unit area of the tissue during embryogenesis.

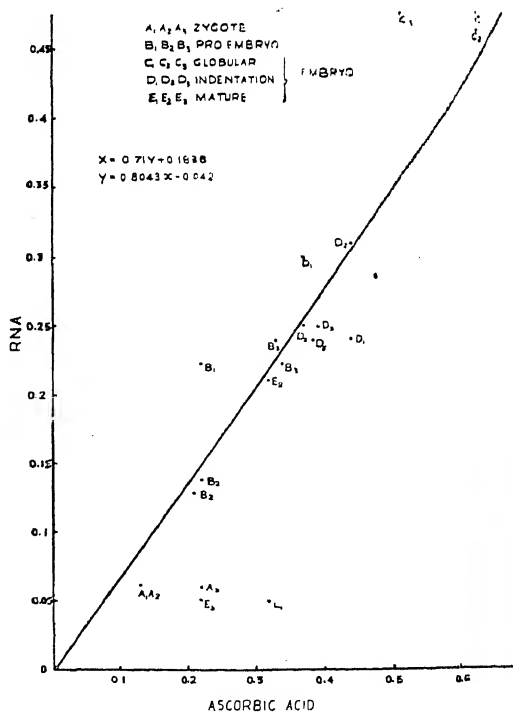


FIG. 2. The regression trend line derived for AA (x-axis) and RNA (y-axis). The slope of the line shows a positive correlation.

and -SH proteins at the zygote ( $A_1 - A_3$ ), globular ( $C_1 - C_3$ ) and indented stage ( $D_1 - D_3$ ) lie (Fig. 2) close to the central trend line while those at the pro-embryo ( $B_1 - B_3$ ) and mature ( $E_1 - E_3$ ) embryos are far from it. Thus a positive correlation amongst the above three metabolites is very sharp at the zygote, globular and indented stages (Fig. 1) while in the rest of the stages, it is comparatively weak.

One of us (P. N. B.) is thankful to the C.S.I.R. for the award of a Junior Research Fellowship.

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## LETTERS TO THE EDITOR

## REACTION OF BIS-DIETHYLDITHIOCARBAMATO ZINC (II) WITH SOME SUBSTITUTED PYRIDINES

As a part of the programme on  $d^{10}$  metal complexes, some zinc (II) complexes with nitrogen and sulphur donor ligands were reported<sup>1-3</sup> earlier from this laboratory. Ligands containing nitrogen as donor atom are bonded strongly to zinc (II) ion, whereas sulphur co-ordination is not very strong. The relative affinities of ligand atoms for different

Infra-red spectra of the compounds were recorded on nujol mulls using a Unicam SP 200 spectrophotometer. Additional bands due to the bonded substituted pyridine ligand were observed over and above the bands due to diethyl dithiocarbamate group clearly indicating that the hetero ligand is definitely bonded to the metal. The compounds are thus few more examples of a less common penta-co-ordination in case of divalent zinc complexes.

TABLE I

Melting point and analytical data of mixed ligand complexes of zinc (II)

Compound Zn (ddtc) <sub>2</sub> L where L =	M.P. (°C)	% Zinc		% Sulphur		% Nitrogen	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
4-Vinyl Pyridine	.. 124	14.01	13.52	27.52	26.82	9.00	8.62
4-Cyano Pyridine	.. 176	14.40	14.58	27.53	26.63	12.33	11.86
4-Amino Pyridine	.. 126	14.34	13.56	28.12	27.54	12.28	11.94
2-Vinyl Pyridine	.. 198	14.01	13.76	..	..	9.00	8.48
2-Amino Pyridine	.. 168	14.34	13.52	..	..	12.28	11.82

acceptor molecules and ions have been summarised<sup>4</sup> by Ahrland *et al.* More recently Mohapatra and Ramana Rao reported<sup>5</sup> some mixed ligand complexes of zinc (II) containing both nitrogen and sulphur donor atoms. In this communication, this work on mixed ligation was extended using a uni-negative bidentate dithiocarbamate ligand and a neutral monodentate substituted pyridine.

Aqueous solutions of zinc sulphate and sodium salt of diethyl dithiocarbamate (ddtc) were mixed in stoichiometric ratio of 1:2 when Zn (ddtc)<sub>2</sub> separated out immediately as a white compound. A chloroform solution of Zn(ddtc)<sub>2</sub> was refluxed with the ligand (L) in chloroform in the ratio 1:1 for two hours when white micro crystalline solids separated out on slow cooling. They were suction filtered and dried. The melting point and analytical data were given in Table I.

It is evident from the analytical data that the compounds have the formula Zn (ddtc)<sub>2</sub>L. They are non-electrolytes in acetone medium and are monomeric in freezing benzene. All the compounds are diamagnetic as expected for a closed shell  $d^{10}$  configuration of the metal ion.

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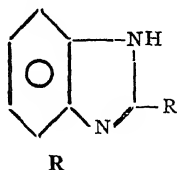
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## ADDITION COMPOUNDS OF ZIRCONYL CHLORIDE WITH BENZIMIDAZOLES

In our earlier work on the complexes of IV group elements with -N- and -O- donor ligands, we have examined the complexes for their structures, and the proposed stereochemistry in majority of the complexes is octahedral<sup>1</sup>.

Now we wish to report in this paper the complexes of  $ZrOCl_2$  with 2-substituted benzimidazoles.



- |                 |                      |
|-----------------|----------------------|
| I. H            | V. $C_6H_5$          |
| II. $CH_3$      | VI. $C_6H_4-CH_2-OH$ |
| III. $C_2H_5$   | VII. $O-HO-C_6H_4$   |
| IV. $C_2H_4-OH$ |                      |

**Experimental.**—The benzimidazoles (I–VII) were prepared according to the methods reported in the literature<sup>3</sup>, and recrystallised from aqueous-alcohol. Anhydrous zirconyl chloride was obtained from  $ZrOCl_2 \cdot 8H_2O$ , by the standard method<sup>3</sup>.

Anhydrous zirconyl chloride (0.01 mole) was dissolved in dry acetone and 0.022 mole of benzimidazole in the same solvent was added were mixed with each other with vigorous shaking. The complex precipitated was filtered, washed with acetone and dried in vacuum over fused calcium chloride.

The complexes were analysed for zirconium, nitrogen and chloride contents by the known methods.

It is evident from the results shown in Table I that zirconium forms two types of adducts. The benzimidazoles I and II form adducts of 1:4 stoichiometry whereas the remaining benzimidazoles (III–VII) form 1:2 adducts.

TABLE I

Analytical data of zirconyl chloride complexes

Lig. No.	Comp. No.	Empirical Formulae	% Zr		% N		% Cl	
			Found	Calcd.	Found	Calcd.	Found	Calcd.
I	VIII	$ZrOCl_2(C_7H_6N_2)_4$	14.37	14.04	16.51	17.23	10.6	10.92
II	IX	$ZrOCl_2(C_8H_8N_2)_4$	13.00	12.92	14.79	15.86	10.49	10.06
III	X	$ZrOCl_2(C_9H_{10}N_2)_2$	18.95	19.40	11.61	11.91	14.47	15.10
IV	XI	$ZrOCl_2(C_9H_{10}N_2O)_2$	18.9	18.17	11.12	11.16	14.10	14.14
V	XII	$ZrOCl_2(C_{13}H_{10}N_2)_2$	16.27	16.11	9.2	9.89	13.08	12.54
VI	XIII	$ZrOCl_2(C_{14}H_{11}N_2O)_2$	15.09	14.56	8.3	8.94	11.59	11.34
VII	XIV	$ZrOCl_2(C_{13}H_{10}N_2O)_2$	15.4	15.25	9.01	9.36	11.36	11.87

The conductance values in DMF at  $10^{-3}$  M are too low to account for any dissociation of the complexes in that solvent.

**Infrared spectra.**—The infrared spectra of the ligands and the complexes in Nujol mull were recorded on a Perkin Elmer infracord-137 B.

The benzimidazoles exhibit a series of bands in the region  $3300-2800\text{ cm}^{-1}$  attributable to the intermolecular hydrogen bonding. These include the CH vibrational modes also. In the benzimidazoles, IV, VI and VII, we observe a weak broad band in the region  $2800-2600\text{ cm}^{-1}$  and is assigned to the intramolecular hydrogen bonded  $-OH$ .

It is rather difficult to assign the band exclusively to the  $C=N$  in these benzimidazoles as the  $NH$ - deformation and  $C=C$  vibrations also appear in the same region  $1625-1500\text{ cm}^{-1}$ . However, in the light of previous data<sup>4</sup>, we have assigned a band of high intensity around  $1613\text{ cm}^{-1}$  to the  $C=N$  stretch.

In all the complexes, we notice that the  $NH$ -stretch shows considerable modification and appears in the region  $3200-3100\text{ cm}^{-1}$ . The  $C=N$  stretch of the complexes appears in the region  $1635-1620\text{ cm}^{-1}$ . These observations imply that the coordination has taken place through the unsaturated nitrogen of the benzimidazole.

In the complexes of the ligands IV and VI, we observe medium intensity bands in the region  $3280-3250\text{ cm}^{-1}$  due to the intramolecular hydrogen bonded  $-OH$ . This shift towards the higher frequency suggests that the coordination through the  $C=N$  has weakened the hydrogen bonding, whereas in the complex of the ligand VII, the broad weak band is observed at  $2740\text{ cm}^{-1}$ .

In view of the previous observations<sup>5</sup>, we have ascribed a high intensity band around  $950\text{ cm}^{-1}$  in the spectra of the complexes to  $Zr=O$  stretch.



The ligands, I and II, form 1 : 4 adducts with  $ZrOCl_2$ , whereas the remaining ligands (III-VII) form 1 : 2 adducts. The bulky groups in the second position of the benzimidazoles (III-VII) may be responsible for this varied behaviour. However, all these observations make us to suggest that in the complexes of the benzimidazoles I and II, zirconium exhibits coordination number seven whereas in the remaining complexes it exhibits coordination number five<sup>6</sup>.

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#### A NEW COLORIMETRIC METHOD FOR THE ESTIMATION OF CARBOFURAN IN PESTICIDE FORMULATIONS

CORBOFURAN (2, 3-dihydro-2, 2-dimethyl-7-benzofuran-1-yl methyl carbamate) is used as an insecticide as well as a nematocide. Gas chromatographic methods are commonly used for the estimation of this compound both in its technical and commercial formulations<sup>1</sup>, and its residues<sup>2-4</sup>. Since most of the analytical laboratories do not possess costly gas chromatograph, it has been difficult to assay this compound. The simple colorimetric method described below is based on the hydrolysis of carbofuran to its phenol under alkaline condition which in turn is allowed to couple with the diazonium salt formed by reaction of sulphanilic acid with

a nitrite, resulting in the production of an orange coloured solution, that obeys Beer-Lambert law.

#### Reagents:

- A.  $NaNO_2$  solution, 0.3% (W/V), freshly prepared in water.
- B. Sulphanilic acid solution, 0.2% (W/V), freshly prepared in 1 N HCl.
- C. Sodium hydroxide, 4 N.
- D. Standard carbofuran solution—100  $\mu$ g/ml—Dissolve 100 mg of analytical standard grade carbofuran (FMC Corporation, New York) in methanol and make upto 1 litre.

#### Procedure

Accurately weigh and transfer 2.0 g of the granular formulations or 0.25 g of wettable powder or technical carbofuran to a 500 ml volumetric flask, add about 100 ml of methanol and agitate well to dissolve the compound. Dilute to the mark with methanol. Filter through a dry filter-paper (Whatman No. 40) taking care to avoid loss of the solvent by evaporation. Transfer 1 ml of the filtrate to a 50 ml volumetric flask. Also transfer 0, 0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 ml of the standard carbofuran (solution D), to a series of 50 ml volumetric flasks. These will give 0, 1, 2, 3, 4, 6 and 8  $\mu$ g/ml of the compound after dilution. Add 5 ml of each of reagents A and B, mix and allow to stand for 30 minutes. Then add 10 ml of reagent C, by means of a fast running pipette and dilute the contents to 50 ml with water. Mix and measure the absorbance of the orange coloured solution after 30 minutes in a suitable spectrophotometer at 490 nm. Construct a calibration curve by plotting the concentration of carbofuran against absorbance (the absorbance values range from 0.092 to 0.770 for the concentration ranges from 1–8  $\mu$ g/ml) and determine the carbofuran concentration in the formulation, by referring to this curve.

The above method has been found to be quite satisfactory for formulations of carbofuran and also for the aqueous solutions containing the compound. However, when the commercial formulations are dissolved in methanol, they should not be exposed to light and preferably, a fresh solution should be used for the estimation. The orange colour of solution obtained has been found to have an absorption maximum at 490–495 nm. The colour is stable even upto 15 hours.

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### SYNTHESIS OF SOME 1-ACYL-4-SUBSTITUTED THIOSEMICARBAZIDES

VARIOUS 1-acyl-4-substituted thiosemicarbazides are known to possess some interesting biological properties like antitubercular<sup>1</sup>, antifungal<sup>2</sup>, hypoglycemic<sup>3</sup> and antiviral<sup>4</sup> activities. Some naturally occurring oxygen heterocyclic compounds such as rotenone, khellin, usnic acid and karanjin well known for their physiological activity contain the furan ring system. Therefore it was deemed desirable to investigate the thiosemicarbazides containing the

furan ring system as they may show pharmacodynamic properties.

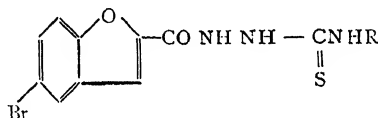
In the present communication fourteen thiosemicarbazides have been prepared for evaluating their biological activity. These were synthesised in the usual way from 5-bromo- and 5,7-dibromo-coumarilic acid hydrazides<sup>5</sup> and appropriate iso-thiocyanates.

*Experimental Section.*—Hydrazide (0.01 mole) and iso-thiocyanate (0.01 mole) were refluxed in 95% ethanol (15 ml) for about 2 hours. The excess of the solvent was distilled off. On cooling, the condensation product separated out which was filtered and recrystallised as usual.

The melting point, yield, molecular formula and analytical data of the prepared thiosemicarbazides are recorded in Tables I and II.

TABLE I

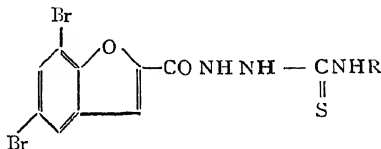
*Characteristics of 1-[5'-bromocoumariloyl]-4-substituted thiosemicarbazides*



Sl. No.	R	Yield %	*M.P. °C	Mol. Formula	%Nitrogen	
					Found	Reqd.
1.	Phenyl	60	152	C <sub>16</sub> H <sub>12</sub> O <sub>2</sub> N <sub>4</sub> SBr	10.68	10.77
2.	4-Chlorophenyl	62	170	C <sub>16</sub> H <sub>11</sub> O <sub>2</sub> N <sub>4</sub> SBrCl	10.02	9.89
3.	4-Methoxy phenyl	65	164	C <sub>17</sub> H <sub>14</sub> O <sub>3</sub> N <sub>4</sub> SBr	10.18	10.00
4.	Cyclo-hexyl	70	198	C <sub>16</sub> H <sub>18</sub> O <sub>2</sub> N <sub>4</sub> SBr	10.45	10.60
5.	4-Bromophenyl	74	174	C <sub>16</sub> H <sub>11</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub>	9.07	8.95
6.	2-Methylphenyl	63	188	C <sub>17</sub> H <sub>14</sub> O <sub>2</sub> N <sub>4</sub> SBr	10.48	10.39
7.	4-Methylphenyl	68	195	C <sub>17</sub> H <sub>14</sub> O <sub>2</sub> N <sub>4</sub> SBr	10.54	10.39

TABLE II

*Characteristics of 1-[5' 7'-dibromocoumariloyl]-4-substituted thiosemicarbazides*



Sl. No.	R	Yield %	*M.P. °C	Mol. Formula	Nitrogen	
					Found	Reqd.
1.	Phenyl	64	171	C <sub>16</sub> H <sub>11</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub>	9.07	8.95
2.	4-Chlorophenyl	68	172	C <sub>16</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub> Cl	8.50	8.34
3.	4-Methoxyphenyl	63	180	C <sub>17</sub> H <sub>13</sub> O <sub>3</sub> N <sub>4</sub> SBr <sub>2</sub>	8.32	8.41
4.	Cyclo-hexyl	65	166	C <sub>16</sub> H <sub>17</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub>	8.68	8.84
5.	4-Bromophenyl	60	202	C <sub>16</sub> H <sub>10</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>3</sub>	7.52	7.66
6.	2-Methylphenyl	70	178	C <sub>17</sub> H <sub>13</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub>	8.77	8.69
7.	4-Methylphenyl	66	184	C <sub>17</sub> H <sub>13</sub> O <sub>2</sub> N <sub>4</sub> SBr <sub>2</sub>	8.59	8.69

\* Melting points are uncorrected.

The antitubercular activity of some of these compounds has been evaluated and some work is still under investigation. This will be published in due course.

The authors wish to record their sincere thanks to Dr. R. S. Kapil, Central Drug Research Institute, Lucknow, for the I.R. spectra and Sri. R. K. Gupta for the assistance during the course of this work.

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#### EFFECT OF BENLATE ON THE GROWTH AND RADIO-ACTIVE ( $^{14}\text{C}$ ) GLUCOSE ASSIMILATION BY *ASPERGILLUS CARNEUS*

BENLATE, Methyl-1-(butyl carbamoyl)-2-benzimidazole carbamate, is a systemic foliar and/or soil applied fungicide active against majority of soil fungi. A fungus, identified as *Aspergillus carneus* (van Teigh) Blochwitz, isolated from soil was reported to tolerate this fungicide upto 10,000 ppm<sup>1</sup>. In this communication, the effect of two different levels of Benlate on the growth and radio-active ( $^{14}\text{C}$ ) glucose assimilation by this fungus are reported.

The fungus was grown in Czapek's broth for 3 days under aerated shake culture. One ml of this was inoculated into each of 99 ml Czapek's broth (with 1% sucrose) in 250 ml Erlenmeyer flasks and were allowed to grow for 12 hr. Thereafter, calculated quantity of Benlate was added to each flask so as to obtain 100 ppm (field rate) and 500 ppm final concentrations of active ingredient of the fungicide. Duplicates were maintained under each treatment and the flasks to which no fungicide was added served as control. After 3 hr of equilibration period 1 ml of uniformly labelled ( $^{14}\text{C}$ ) radio-active glucose (aqueous solution) of specific activity 0.01 mc/ml (22 mc/mM of glucose) was added to each flask through an automatic micro-syringe and incubated again for 5 hr. The fungal mat was then recovered by filtration through Whatman No. 1 filter-paper after several washings with normal saline and the total yield (moisture-free) of the fungus was found out. A portion of the yield was taken in a stainless steel planchet and the

radio-activity monitored with a gas flow proportional counting system (Electronic Corporation of India Ltd.).

A known weight of the fungal growth was extracted for 30 min. with 25 ml of 80% boiling ethanol. The volume of the extract was reduced to 5 ml in a flash evaporator before passing it through Dowex-1 and Dowex-50 ion-exchange resin columns in succession. The neutral fraction (effluent) of the extract was separately collected while the Dowex-1 and Dowex-50 resins were eluted with 2 N HCl and NaOH respectively, to obtain the basic and acidic fractions of the alcohol extract. Appropriate quantity of each fraction was monitored for radioactivity and the specific activities were computed on moisture-free basis.

The mycelial yield of *A. carneus* and the incorporation of ( $^{14}\text{C}$ )-radioactivity in the cells were significantly increased by Benlate treatment (Table I).

TABLE I

Effect of Benlate on growth and radioactive ( $^{14}\text{C}$ ) glucose assimilation by *Aspergillus carneus*

Treatment	Total mycelial yield (mg)	Radio-activity assimilated (CPM/100 mg)
No fungicide (control) ..	439.5	3774.30 $\pm$ 26.87
100 ppm Benlate ..	518.5	4020.30 $\pm$ 107.71
500 ppm "	542.5	4433.00 $\pm$ 253.38

Radioactive glucose assimilation in the basic and acidic fractions in the alcohol soluble portion of the fungal matter have also been increased significantly by the fungicide treatment while the incorporation in the neutral fraction of the extract has been reduced (Table II). There was also a notable increase in the assimilation of radioactive carbon in the alcohol-insoluble matter of the fungus treated with Benlate at 500 ppm level.

The enhanced growth and radio-carbon assimilation of *A. carneus* in the presence of Benlate suggests a possible stimulation of the carbon assimilation process in the fungus. Tweedy and Loeppky<sup>2</sup> have reported an inhibition in 'hexose monophosphate shunt' pathway and the rate of glucose catabolism by Atrazine, a herbicide, treatment in *Fusarium oxysporum* f. *moniliforme*. But, the increase in radio-carbon content in the basic and acidic fractions with a concomitant decrease in the neutral fraction of the alcohol extract, presumably composed of simple sugars, as well as the increase in specific activity of the insoluble residue of the

TABLE II

Effect of Benlate on radioactive ( $^{14}\text{C}$ ) glucose assimilation in the alcohol soluble fractions of *Aspergillus carneus*

Treatment	Activity in insoluble residue (CPM/100 mg)	Activity in fractions (% of total alcohol soluble activity)		
		Basic	Acidic	Neutral
No fungicide (Control)	.. 588.15 $\pm$ 15.59	14.05	15.06	70.89
100 ppm Benlate	.. 616.65 $\pm$ 38.75	31.28	26.17	42.60
500 ppm ..	.. 1073.38 $\pm$ 4.28	23.30	17.85	58.85

fungal matter supports the view that Benlate altered the glucose metabolism of *A. carneus* in favour of carbon assimilatory processes. The exact mechanism of action is yet to be worked out.

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### THE INTERACTION BETWEEN DISULPHUR DICHLORIDE AND IODINE MONOCHLORIDE

DISULPHUR dichloride occupies a position of special interest in sulphur chemistry<sup>1</sup>. In the course of the investigation of the reaction between iodine monochloride and various sulphur compounds<sup>2</sup>, it was considered worthwhile to study the reaction between  $\text{S}_2\text{Cl}_2$  and  $\text{ICl}$  in 5N HCl medium.

#### MATERIALS AND METHODS

**Reagents.**—Stock solutions of iodine monochloride in 5N HCl were prepared and standardised by the procedure described earlier<sup>2</sup>. Dilute solutions of  $\text{S}_2\text{Cl}_2$  in dry carbon tetrachloride were prepared and their strengths were checked by standard methods<sup>3</sup>. All other reagents employed were of analytical reagent grade.

**Procedure.**—Known volumes of the  $\text{ICl}$  solution (50 ml) were taken in 500 ml stoppered conical flasks. Measured aliquots of the  $\text{S}_2\text{Cl}_2$  solution were added to these in a thin stream, with vigorous mixing. The flasks were stoppered and the contents were given a top to bottom shaking for about 10 minutes and were allowed to stand for an hour, with frequent vigorous shaking. 20 ml

$\text{KI}$  solution (20%) was added and the liberated iodine was titrated with standard sodium thiosulphate solution. (Blanks were run concurrently; no blank correction was necessary.)

In a few experiments, the iodine liberated was separated by extraction with  $\text{CCl}_4$ . The iodine in the  $\text{CCl}_4$  layer as well as the unreacted  $\text{ICl}$  in the aqueous layer were separately determined by adding excess aqueous  $\text{KI}$  and titrating the liberated iodine with standard sodium thiosulphate. The utility of such an alternative procedure is discussed elsewhere<sup>4</sup>.

#### RESULTS AND DISCUSSION

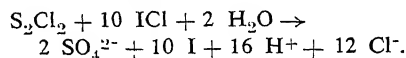
Typical results are presented in Table I. It may be seen from the table that one mole of  $\text{S}_2\text{Cl}_2$

TABLE I

Oxidation of disulphur dichloride with iodine monochloride

Expt, No.	$\text{S}_2\text{Cl}_2$ taken (mmol)	$\text{ICl}$ consumed (mmol)	Moles $\text{ICl}$ consumed per mole of $\text{S}_2\text{Cl}_2$
1	0.09294	0.9292	9.997
2	0.1859	1.849	9.922
3	0.04647	0.4793	10.31
4	0.06970	0.7091	10.18

reacts with 10 moles of  $\text{ICl}$  in accordance with following reaction scheme :



This simple reaction could be used for a convenient quantitative determination of disulphur dichloride.

The author wishes to express his grateful thanks to Dr. C. G. R. Nair, Department of Chemistry, Kerala University, for his keen interest and helpful suggestions.

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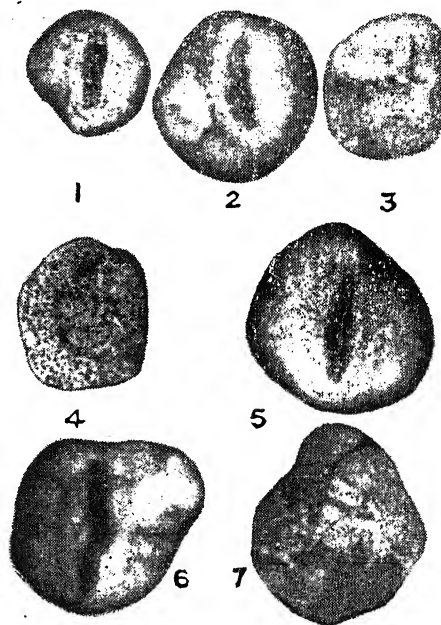
#### OCCURRENCE OF *INDICOLA RAJASTHANENSIS* IN THE LUTETIAN ROCKS OF VINJHAN MIANI AREA, SOUTHWESTERN KUTCH, GUJARAT

SINGH and Kalia (1970) erected a new family Indicolidae in the Superfamily Globigerinacea to receive a new planktonic foraminiferal form *Indicola rajasthanensis* Singh and Kalia, characterized by an interiomarginal multiple primary aperture and a peculiar structure developed in the umbilical ellipsoidal cavity. They reported this form from the *Flosculina* Zone of Bikaner Stage of Kirthar Series (Lutetian) of Rajasthan. The *Flosculina* Zone, according to them, is equivalent to Bolli's topmost Lutetian Zone (*Truncorotaloides rohri* Zone) in Trinidad.

Middle Eocene of Rajasthan is, according to Singh and Kalia (1970), divisible into the lower Marh Stage (unfossiliferous) and the upper Bikaner Stage (fossiliferous). Bikaner Stage is divisible into the lower *Discocyclina* Zone and the upper *Flosculina* Zone, and it is within the latter that *Indicola rajasthanensis* is restricted.

In Vinjhan-Miani, *Indicola rajasthanensis* has been observed in the Lutetian rocks which occur as thin patches of yellow limestone and marls. Unlike the Lutetian of Rajasthan the Lutetian rocks of the Vinjhan-Miani area, are not distinguishable into similar biozones because *Discocyclina* and *Fasciolites* (*Flosculina*) both are found here uniformly occurring together throughout the Lutetian strata. Any attempt at zonation of these beds in the area based on larger foraminifers is thus turned futile because of poor thickness of the strata and occurrence together of such forms throughout the horizon.

The planktonic foraminifers recovered from the Lutetian rocks here, however, appear to correlate these strata with Mohan and Soodan's *Globigerinoides kugleri*—*Globigerina frontosa* assemblage Zone and *Orbulinoides beckmanii* Zone (1970). Their former zone is equivalent of the combined *Globigerina frontosa* and *Truncorotaloides topilensis* Zones of Bandy (1964) and *Globigerapsis kugleri* and *Globorotalia lehneri* Zones of Bolli (1967). The *Orbulinoides beckmanni* Zone, on the other hand, is equal to *Porticulasphaera mexicana* Zone of Bolli (1957) and Bandy (1964). In the association of such planktonic foraminifers (mentioned later), *Indicola rajasthanensis* occurs as a rare form (50 g material of the sample has yielded only 9 specimens).



FIGS. 1-7. *Indicola rajasthanensis* Singh and Kalia. Figs. 1-2, 5-6. Umbilical view showing the elliptical umbilical cavity. Fig. 3. Lateral view showing the multiple interiomarginal apertures placed on the open ends of small tubes; Figs. 4, 7. Spiral view showing the coarsely perforated test. Fig. 5. Umbilical view showing the median thin partition in the elliptical umbilical cavity,  $\times 81$ .

The sparse occurrence of *Indicola rajasthanensis* in a low frequency in Vinjhan-Miani seems quite distinct from that in the original locality where it commonly occurs in *Flosculina* Zone, equivalent to *Truncorotaloides rohri* Zone, and is missing in the underlying *Discocyclina* Zone, correlated with *Globorotalia lehneri* Zone.

As compared to the Kirthar (Lutetian) outcrops exposed around Lakhpat, Beranda-Bernana and other adjoining localities of Kutch and the Kirthars (Lutetian) of Rajasthan, Kirthars of Vinjhan-Miani have very scanty outcrops and their thickness is very small. Their poor development in Vinjhan-Miani is attributed to the shallow nature of the Kirthar sea here. On the contrary, the Kirthar sea was quite deep enough to have allowed deposition of thick strata in the areas (mentioned above) other than the present area. As a result, thick Lutetian sediments of these areas are seen pinching out in the Vinjhan-Miani area. The shallowness of sea also accounts for much of the mixing up of microfauna (larger foraminifera), especially *Fasciolites* and *Discocyclina*, which in comparatively deeper parts of the sea (e.g., Rajasthan) must have preferred separate ecological niches.

After comparing the occurrence of *Indicola rajasthanensis* at both the places, the following conclusions are reached :

(i) *Indicola rajasthanensis* appears to be a Lutetian form.

(ii) Its scanty appearance in the Lutetian strata of Vinjhan-Miani, ranging from *Globigerapsis kugleri* Zone to *Porticulasphaera mexicana* Zone, is a pointer to the fact that it originally evolved much earlier in the Middle Eocene (Lutetian) times, and was later dispersed throughout the Kirthar sea.

(iii) As the earlier Lutetian times were the period when it was just evolving, it is scantily represented in the strata of these times. It is believed that it continued to be so until the beginning of the to most Lutetian when it firmly established itself and found a niche in the association of *Fasciolites*.

(iv) Its absence in the underlying *Discocyclina* Zone of Lutetian of Rajasthan and its presence in the Vinjhan-Miani area of Kutch in the strata, also equivalent to this underlying zone of Rajasthan, seem to suggest that its actual home locality lay somewhere in Kutch from where it was introduced into the regions of Rajasthan in late Middle Eocene times.

(v) Its preference in Rajasthan, where it seemed to have flourished, for a particular biotope in which only few larger and smaller forms could settle well would suggest that *Indicola rajasthanensis* was much sensitive to an environment where much of the mixed fauna occurred and where many environmental stresses, as a consequence, operated together (e.g., Vinajhan-Miani, Kutch).

In association with *Indicola rajasthanensis*, the following planktonic forms have been found :

*Globigerina officinalis*, *G. turgida*, *G. linaperta*, *G. senilis*, *G. ouachitaensis ouachitaensis*, *G. veguaensis*, *G. galavisi*, *G. eocaenica*, *G. pseudoeocaena pseudoeocaena*, *G. venezuelana*, *G. bacuana*, *G. frontosa*, *G. sp.*, *Globigerinoides rubrifformis*, *G. higginsii*, *Glogigerapsis kugleri*, *Globorotalia opina nana*, *G. broedermanni*, *G. crassata*, *G. spinuloinflata*, *G. spinulosa*, *Turborotalia centralis*, *T. cerroazuensis*, *Globigerinatheka barri*, *Inordinatosphaera indica*, *Globanomalina micra*, *Chiloguembelina tenuis*, *Truncorotaloides rohri*, *T. topilensis* and *T. sp.*

In addition to this, the author has also recorded much diversified benthonic foraminifera in large numbers. A few important ones which have been identified upto generic level only may be mentioned here as follows : *Pararotalia*, *Rotalia*, *Epistomaria*, *Planulinoides*, *Bigenerina*, *Glabratella*, *Cymbaloporella*, *Miliola*, *Fissurina*, *Oolina*, *Clavulina*, *Heterolepa*, *Epistominella*, *Lagena*, *Anomalinoides*, *Discorbis*, *Bolivina*, *Textularia*, and *Quinqueloculina*.

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#### OCCURENC OF HORNBLENDEGARBEN-SCHIEFER IN MANGALUR AREA, GULBARGA DISTRICT, KARNATAKA

A BELT of hornblende schists, occupying an area of about 20 sq km, is enclosed in the Peninsular Gneiss in the area around Mangalur in the Gulbarga District of Karnataka (Mukherjee, 1941). During the course of study of the schists and associated rocks, it was found that the schists, at places, have developed conspicuous porphyroblastic structure resulting into an unusual type of rock. On detailed

examination this rock was found to have characters typical of *hornblendegarbenschiefer*. This note records, for the first time, the occurrence of such a rock in this area and describes its mode of occurrence and petrological characters.

The rock is found to be present at four different localities within a distance of 3 km to the south, south-west, north and east of Mangalur. The hornblende schists in this area strike NNW-SSE and dip 60° towards WSW. In all the four localities the schists have been intruded by pegmatite dykes, of varying width and length, along the planes of schistosity. In one locality the pegmatitic material has been injected in lit-par-lit manner. The hornblende schists have developed large porphyroblasts of hornblende in the contact zones of the pegmatites in all these localities.

The rock, in hand specimen, shows different stages of development of porphyroblastic structure. In the earlier stages the small porphyroblasts are found to have developed along and across the planes of schistosity without much disturbing the schistosity. With the increase in the number and size of the porphyroblasts (5 mm in length) the rock becomes more coarse grained and the schistosity is interrupted. In such rocks the fine grained whitish pegmatitic material is seen between the planes of schistosity. In the later stages the number and size of the porphyroblasts are considerably increased. The rock loses its original schistosity, but due to the partial retention of parallel arrangement of hornblende needles, looks like a gneiss. In some cases it develops granoblastic structure, the porphyroblasts forming the bulk of the rock with finer grained matrix occupying the interstices. The weathered surfaces of such rocks acquire a knotted appearance, the black knots representing either individual porphyroblasts upto 1 cm in length or aggregates of smaller porphyroblasts.

In thin sections the rock is found to be mainly composed of hornblende, quartz and feldspar with biotite, epidote and zoisite as accessories. In the earlier stages of development of the structure the rock shows a parallel arrangement of hornblende needles representing the planes of schistosity, which are separated by layers rich in quartz and sericitized plagioclase. Knots of larger porphyroblasts of hornblende are found to be developed, around which the hornblende needles or quartz-sericite matrix is found to be curving. In other cases, the porphyroblasts have grown to larger size at the expense of the matrix and the growth of such porphyroblasts can be very clearly seen from the serrated ends of such grains. In some cases relicts of the matrix are found inside the porphyro-

blasts. In some of the porphyroblasts different stages of growth have been documented by the inclusions of well marked transverse sections of hornblende inside the larger hornblende grains. The two types of hornblende grains mentioned above show different optic orientation, refractive index, pleochroism and birefringence indicating the change in the composition of hornblende during its growth. At times numerous flakes of biotite are found in large porphyroblasts of hornblende giving rise to poikiloblastic structure. In cases where the porphyroblasts have grown to larger size they show rounding. In such cases the matrix is found to be swirling around them. The majority of the porphyroblasts show polysynthetic twinning. The hornblende is mostly actinolitic with very faint pleochroism from almost colourless to pale bluish green.

The megascopic and microscopic characters of the rock distinguish it from the commonly occurring hornblende schists in the area. The most remarkable character of the rock is the development of the porphyroblasts which imparts to it spotted or knotted appearance. On the basis of this characteristic structure the rock has been identified as *Hornblendegarbenschiefer* (Harker, 1950, p. 202; Spry, 1969, p. 269). According to Spry, "the texture is due to pronounced post-tectonic mimetic crystallisation of the amphibole". In the present instance, it is found that the structure is developed, in all the cases, in the contact zones of pegmatite dykes. It, therefore, appears that the development of large porphyroblasts of hornblende in the hornblende schists is a result of over-print of the later thermal metamorphism on the schists due to the intrusion of pegmatite in them.

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#### OXYGEN CONSUMPTION OF *CHELA BACAILA* (HAM.) IN RELATION TO SIZE

THE metabolic rates of animals are dependent on surface area or weight<sup>1-3</sup>. In many fishes the metabolic rate decreases with increasing weight<sup>4-7</sup>. However, Bertalanffy<sup>8</sup> recognised three types of metabolic rates, viz., surface area dependent, directly proportional to weight or intermediate between

proportionality to weight and surface area. The present investigation is to find out the metabolic rate and its dependence on weight in the freshwater fish *Chela bacaila*.

The fishes were collected from Godavari river near Nanded and were kept for about a week in a large tank and then in aquaria for at least two days under laboratory conditions. They were fed with beaten rice and hydrilla. A four-neck all-glass respiratory chamber, painted black from outside was used. Oxygen consumption was measured for three continuous hours by drawing samples of water every hour. Average of these three readings was taken as the routine metabolism of the fish. Every day the experiments were started at 4 p.m. to avoid the effect of diurnal rhythms, if any<sup>9</sup>. Dissolved oxygen was estimated by Winkler's method<sup>10</sup>.

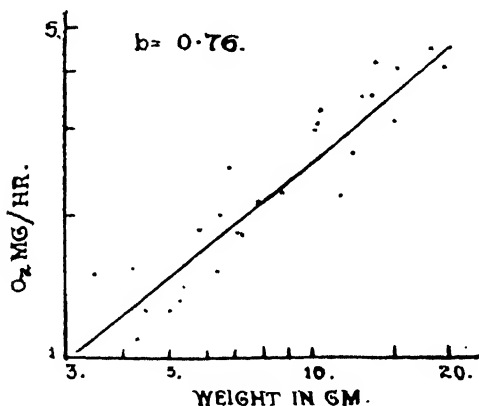


FIG. 1. Oxygen consumption of *Chela Bagaila* in relation to size.

The results are represented as in Fig. 1. The  $a$  and  $b$  values obtained from the statistical analysis of the data for the formula  $M = a W^b$ , where  $M$  is metabolism,  $W$  is weight and  $a$  is  $Y$  intercept are 0.4655 and 0.76 respectively. Therefore the equation becomes

$$\log M = \log 0.4655 + 0.76 \log W.$$

Since the  $b$  value obtained is more than  $2/3$ , the metabolic rate does not fit into Rubner's surface law, nor it is directly proportional to the weight. However the metabolic rate agrees with the third type of Bertalanffy<sup>8</sup>. This is in accordance with the earlier findings<sup>4-7</sup>.

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#### CYTOLOGY OF *ORYZA* *OFFICINALIS* (4n) × *O. LATIFOLIA*

A SEMI-STERILE  $F_2$  plant, out of a natural cross between *Oryza officinalis* ( $2n=24$ , 12 II) of Ceylon and another of Bangkok, was treated with colchicine and an intervarietal tetraploid plant was obtained by Gopalakrishnan and Shastry<sup>1</sup>. One of the present authors (S. D. S.) crossed this tetraploid with *O. latifolia* ( $2n=48$ , 24 II) of Costa Rica (CR-1). The cytology of this hybrid is reported here.

For cytological studies, acetic alcohol (1:3) fixative and aceto-carmine stain were used. Details of chromosome configurations are provided in Table I. In most of the cells, either 24 II or 22 II + 1 IV were noticed.

Morinaga and Kuriyama<sup>2</sup> analyzed the genomic constitution of *O. officinalis* (Ceylon) and proposed its genome as CC. If the Bangkok collection of *O. officinalis* is also CC, then the observation of 12 II in the intervarietal diploid hybrid, *O. officinalis* (Ceylon) × *O. officinalis* (Bangkok) by Gopalakrishnan<sup>3</sup> would be normal C-C pairing. In the intervarietal tetraploid (CCCC) Gopalakrishnan<sup>3</sup> and Hu<sup>4</sup> observed mostly bivalents and explained it as due to cryptic structural differences between chromosomes of the two species<sup>3</sup> or due to genetic mechanisms suppressing quadrivalent formation<sup>4</sup>. The hybrid between this tetraploid *O. officinalis* (CCCC) and *O. latifolia* (CCDD) would, therefore, be CCCD. Theoretically, one may expect 12 III + 12 I in this hybrid. The present observation of 24 II in this material, therefore, deserves explanation.



TABLE I

Pairing of chromosomes at diakinesis and  
metaphase-I in the hybrid,  
*Oryza officinalis* (4n) × *O. latifolia*

Configurations			No. of PMCs observed
IV	II	I	
	24		10
	23	2	2
1	22		19
1	21	2	2
2	20		13
1	20	4	1
2	19	2	2
1	19	6	2
	19	10	1
4	16		6
5	13	2	1
Total			59
Range 0-5	13-24	0-10	
Mean 1.41	20.85	0.68	

Morinaga<sup>5</sup> analysed *O. sativa*, *O. minuta* and *O. latifolia* and suggested AA, BBCC and CCDD respectively as their genomes. This led to the presumption that diploid BB, CC and DD species also must occur (or have occurred?) in nature. Of these, *O. officinalis*, having CC genome, has been well established but the DD species is still not identified. However, information regarding the interrelationship of C and D genomes has been gathered from the cytological studies of hybrids involving CCDD genome. The present observation on *O. officinalis* (4n) × *O. latifolia* throws some more light on the relationship of C and D genomes.

Richharia<sup>6</sup> and Sampath<sup>7</sup> proposed that the genomes C and D are homeologous and suggested subgenomic symbols O<sup>1</sup> and O<sup>2</sup> respectively for them. In CCDD species (*latifolia*, *alta*, *grandiglumis*), one gets mostly bivalents, but the maximum pairing observed in six ACD hybrids ranged from 4 to 11 bivalents and in two CDE hybrids from 11 to 12 bivalents<sup>8,9</sup>. Li<sup>10</sup> presumed that 'majority of these bivalents result from pairing between chromosomes of C and D genomes'. Gopalakrishnan<sup>3</sup> observed upto 12 bivalents in a CDE hybrid and could identify most of them as due to C-D pairing. Gopalakrishnan and Sampath<sup>11,12</sup>, therefore, concluded that D genome is a variant of C genome but their pairing in triploid and tetraploid combinations is under genic control. However, in all these hybrids a third alien genome (A or E) is also present which, to some extent, takes part in the pairing and thus makes analysis of the different intergenomic pairings difficult. The

present hybrid, in this respect, is better suited to determine the extent of C-D pairing as it lacks any other genome.

In tetraploid *O. officinalis* (CCCC) as well as in octoploid *O. minuta* (BBBBCCCC) and *O. latifolia* (CCCCDDDD), the number of observed quadrivalents are fewer (mean 3.71, 7.26, 4.11 respectively) than expected<sup>4,13,14</sup>. This indicates that genes are present which suppress intra-genomic pairing, especially higher than bivalents. Since the number of quadrivalents was least in the case of CCCCDDDD plant, it is suspected that D genome might be suppressing C-C pairing<sup>15</sup> especially associations higher than bivalents. The observation of about 24 II in the present hybrid could, in that case, be explained as 12 II due to C-C pairing and the other 12 II due to C-D pairing.

An alternative hypothesis would be that the genomic constitution of *O. officinalis* (Bangkok) is DD. If so, the genome of the hybrid *O. officinalis* (Ceylon) × *O. officinalis* (Bangkok) would be CD, and the 12 II observed by Gopalakrishnan<sup>3</sup> in this hybrid could be due to inter-genomic (C-D) pairing. The intervarietal tetraploid would be CCDD which forms mostly bivalents as a result of genic control<sup>11</sup> or diploidizing genes<sup>14</sup> or preferential pairing due to differential affinity<sup>3</sup>. In that case, the hybrid *O. officinalis* (4n) × *O. latifolia* would also be CCDD and form 24 II as observed in the present study. Though such a hypothesis offers a simpler explanation for these cases, it not only demands re-examination of all earlier observations but also further experimental confirmation.

According to Gopalakrishnan<sup>3</sup>, there is no necessity for searching the plants with D genome outside the species *O. officinalis* as one or the other geographical race of this species may possibly contain it. It is, therefore, possible that *O. officinalis* (Bangkok) might contain DD genome.

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# **EFFECT OF TEMPERATURE AND SUNLIGHT ON THE INFECTIVITY OF A NUCLEAR POLYHEDROSIS VIRUS OF *SPODOPTERA* *MAURITIA* (BOISDUVAL)**

INSECT viruses are known to be inactivated by extreme temperatures, sunlight and ultraviolet rays. Information on the influence of such factors on viral infectivity is essential for judging the suitability of a viral pathogen for pest control. The present investigations were taken up to study the effect of temperature and sunlight on the infectivity of a nuclear polyhedrosis virus (NPV) of *Spodoptera mauritia*.

Second instar larvae reared in the laboratory on the common grass, *Ischaemum aristatum* H., were used in all tests. A purified suspension of polyhedra isolated from diseased larvae of *S. mauritia* formed the infective material. Larvae were inoculated as follows: Two topmost leaves of tender grass terminals were smeared with the polyhedral suspension to get a thin film of inoculum on both sides of the leaves. The contaminated leaves were air-dried and two larvae each were released over a terminal in separate specimen vials. Control larvae were provided with untreated leaves. After 24 hours the larvae were transferred to fresh foliage and reared individually.

Seven 1-ml aliquots containing  $5 \times 10^8$  polyhedra/ml were heated in a water-bath at 40, 50, 60, 70, 80, 90 and 95°C for 10 minutes. After heat treatments the suspensions were cooled and fed to 25 larvae each as described above.

Eight 1-ml aliquots of virus suspension containing  $5 \times 10^8$  polyhedra/ml were poured into petri dishes and air-dried. The polyhedra were then exposed to direct sunlight in open dishes for 1, 5, 10, 15, 24, 48, 72 and 96 hours. After exposure the samples were resuspended in distilled water to get the original

concentration. Each sample was fed to 25 larvae as outlined earlier.

TABLE I

*Effect of temperature on infectivity of the NPV of S. mauritia*

Temperature, °C	Incubation period in days (mean)	% Mortality due to polyhedrosis	% of Mortality due to other causes	% Pupation
40	3.1	100	Nil	Nil
50	3.6	100	..	..
60	3.6	100	..	..
70	3.8	100	..	..
80	4.1	92	..	8
90	4.0	82.8	3.4	13.8
95	..	Nil	4	96.0

The results (Table I) show that heating of the virus upto 70°C did not affect the final percentage mortality. But on exposure to 80°C a reduction in the rate of mortality was observed. The infectivity reduced still further on exposure to 90°C and there was no larval mortality due to polyhedrosis when the larvae were treated with virus heated to 95°C. These observations indicate that the thermal inactivation point (TIP) of the virus, when heated for 10 minutes, lies between 90 and 95°C. The TIP of the NPV of *Spodoptera litura* was also between 90 and 95°C<sup>1</sup>. The NPV of *Heliothis* withstood exposure to 60°C for 2 hours and was completely inactivated at 93.3°C in 1 hour<sup>2</sup>. The NPV of *S. mauritia* is not as heat tolerant as the *Heliothis* virus.

TABLE II

*Effect of sunlight on the infectivity of the NPV of S. mauritia*

Period of exposure (hours)	Incubation period in days (mean)	% Mortality due to polyhedrosis	% mortality due to other causes	% Pupation
1	3.2	96	4	Nil
5	3.1	100	Nil	..
10	3.2	100	..	..
15	3.1	100	..	..
24	3.1	100	..	..
48	3.4	100	..	..
72	4.0	60	..	40
96	4.0	8	..	92

The observations (Table II) reveal that the virus could withstand exposure to sunlight for 48 hours without loss of infectivity. It retained substantial infectivity even after 72 hours of exposure but was almost non-infective after 96 hours. *Heliothis* virus applied to cotton foliage lost most of its viral activity after a day<sup>3</sup>. Morris<sup>4</sup> observed that infectivity of NPV of *Lambdina fiscellaria somnaria* was

reduced considerably after exposure for 5 hours, the persistence after 35 hours being only 11%. The present findings suggest that NPV of *S. mauritia* is more tolerant to sunlight than *Heliothis* and *Lambda fuscicellaria somniaria* viruses.

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#### OVIPOSITION BEHAVIOUR OF *HELIOTHIS ARMIGERA* (Hbn.) IN COTTON, HYBRID-4

*Heliothis armigera* (Hbn.) (Lep.: Noctuidae) is an important pest of cotton causing severe damage to bolls if proper insecticidal treatments are not given in time. During September 1971, some fields of cotton (Hybrid-4 variety) at the farms of the Gujarat Agricultural University, Anand Campus, generally developed yellowish-green crop with sporadic normal deep-green plants. On the other hand, in most of the other fields the crop was very luxuriant with the plants having only deep-green leaves. Frequent examination of the crop in different fields showed that *H. armigera* eggs were laid in larger numbers on the deep-green leaves in luxuriant crop than on the leaves

of yellowish-green crop. This visual observation confirmed by recording the numbers of eggs laid on the upper and lower surfaces of each of the tender, medium and older leaves in both the upper and lower halves of each plant. The types of plants observed were pale-green and deep-green in the yellowish-green fields and deep-green plants in the luxuriant crop. 50 plants of each type were examined from each of the three yellowish green fields selected and from each of the two luxuriant deep-green fields.

The results (Table I) were analysed statistically and it was found that irrespective of the crop condition, the moths definitely preferred for oviposition: (1) the upper half of the plant to the lower half, (2) the upper surface of the leaf to the lower surface and (3) the tender leaves to medium and old leaves. In other words, maximum eggs were laid on the upper surface of the tender leaves present at the upper half of the plant. McColloch (1920)<sup>1</sup> who studied the oviposition behaviour of the corn earworm, *Heliothis zea* (Boddie) on corn also found that the moths preferred the upper surface for egg-laying.

The data further revealed that in a yellowish-green crop, more eggs were deposited on the deep-green plants than on the yellowish-green ones. However, on comparing this result of oviposition with that of the deep-green plants in the luxuriant deep-green crop, the latter is found to be the most preferred. This shows that the moths have a marked attraction for dark-green colour.

Study of various interactions revealed that the moths preferred the medium leaves of luxuriant crop to tender leaves of yellowish-green crop, upper half of yellowish crop to lower half of luxuriant crop and tender leaves of lower half of the luxuriant crop to medium leaves of upper half of the yellowish-green crop as far as their oviposition behaviour is concerned.

TABLE I

Mean number of *H. armigera* eggs per 50 leaves of yellowish-green and deep-green plants of cotton (Hybrid-4 variety) in two types of fields

Crop condition	Type of leaf	Yellowish-green plants				Deep-green plants			
		Upper half		Lower half		Upper half		Lower half	
		U.S.	L.S.	U.S.	L.S.	U.S.	L.S.	U.S.	L.S.
Yellowish-green	Tender	8.3	1.3	3.0	0.3	19.0	1.3	6.6	1.6
	Medium	2.0	0.6	0.0	0.0	8.3	2.0	3.0	1.0
	Old	1.3	0.0	0.0	0.0	4.0	1.0	1.0	0.3
Deep-green	Tender	No plants of this type in the crop				57.5	15.0	15.5	6.0
	Medium					24.0	14.5	4.0	3.5
	Old					5.5	3.0	0.5	1.5

U.S.—Upper surface; L.S.—Lower surface.

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# IAA SYNTHESIS IN RELATION TO PHYLLOSPHERE MICROFLORA OF *SOLANUM MELONGENA* L. SPRAYED WITH PESTICIDES

It is well known that application of pesticides on the foliage has profound influence on the phyllosphere microflora<sup>1</sup>. The epiphytic microorganisms play a major role in the production of indole acetic acid<sup>2</sup>. The present communication reports on the production of indole acetic acid in relation to phyllosphere microflora of brinjal (*Solanum melongena* L.) sprayed with endrin and parathion.

The results indicated that, in general, there was a marked reduction in bacterial, fungal and actinomycetes populations 24 hr after spray of insecticides whereas the reduction was less apparent 20 days after spray (Table I). A decrease in IAA production potential in the phyllosphere region corresponding to a decrease in microbial population due to the spray of the insecticides, besides confirming their toxic effect on the microbes<sup>3</sup>, supports the view that the phyllosphere microflora are essentially responsible for the production of IAA<sup>2,6</sup>. A slight increase in IAA production 20 days after spray might be due to stimulation of particular group of organisms<sup>7</sup>.

Specific stimulation of certain fungal genera due to application of insecticides like aldrin, parathion and carbaryl was reported earlier<sup>5,8</sup>. Such stimulation may be due to the presence of IAA since it was reported that IAA was known to break the dormancy of fungal spores under *in vitro* conditions<sup>9,10</sup>. In the present study, a general decrease in fungal population with a concomitant reduction in IAA production potential on the leaves, 24 hr after spray of insecticides suggested that the IAA

TABLE I

Variation in the phyllosphere microflora and IAA synthesis as influenced by foliar spray of endrin and parathion

Treatment	Microbial population						IAA synthesis ( $\mu\text{g}/100 \text{ cm}^2$ )	
	Bacteria ( $\times 10^4/100 \text{ cm}^2$ )		Actinomycetes ( $\times 10^3/100 \text{ cm}^2$ )		Fungi ( $\times 10^2/100 \text{ cm}^2$ )		24 hr	20 days
	24 hr	20 days	24 hr	20 days	24 hr	20 days		
Endrin 0.02%	.. 1848.63	451.59	25.78	4.92	56.58	63.80	46.58	38.12
Parathion 0.05%	.. 1644.92	430.90	14.13	4.92	25.78	81.66	41.69	43.29
Control	.. 3344.55	442.64	44.20	5.41	54.40	111.46	296.38	37.40

Two months old brinjal crop was sprayed with endrin (1, 2, 3, 4, 10-hexachloro-6, 7-epoxy-1, 4, 5, 6, 7, 8, 8  $\alpha$ -octahydro-1, 4-exo-5, 8-exodimethanonaphthalene) 0.02% and parathion (dimethyl *p*-nitrophenyl thiophosphate) 0.05%. The populations of bacteria, actinomycetes and fungi in the phyllosphere region were estimated 24 hr and 20 days after spray<sup>3</sup> and the IAA production potential in the phyllosphere region was estimated by immersing the whole leaves in 100 ml of 0.1 M phosphate buffer (pH 7.0) supplemented with 0.005 M DL tryptophan and 5% sucrose. Quantitative estimation was done employing Salper's reagent and the presence of IAA was confirmed by paper chromatography using Salkowski spray reagent<sup>4</sup>.

pool in the phyllosphere region might play an important role in the development of fungal diseases.

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### RHIZOSPHERE MYCOFLORA OF HEALTHY AND INFECTED JUTE PLANTS

THE subject of rhizosphere populations in soil, surrounding roots of higher plants, has attracted considerable attention in recent years. From the literature it is evident that there is an increase in number of fungi in the rhizosphere region than that present in the soil distant from the plant roots<sup>1-3</sup>. Increased concentration of mycoflora in the root regions of diseased plants has been reported by various workers<sup>4,5</sup>. In the present investigation the rhizosphere fungi of *Macrophomina phaseoli* (Maubl.) Ashby infected jute plants (*Corchorus capsularis* L.) have been compared with those of healthy ones.

Healthy and *Macrophomina*-infected seeds of *Corchorus capsularis* were sown in the Burdwan University field in the first week of May 1973. Typical symptoms of disease developed after germination in some of the plants. Rhizosphere soil samples from five plants, each of healthy and *Macrophomina*-infected ones were collected at regular intervals with sterile spatula. The soil samples were assayed by the dilution plate method using modified Martin's medium. The plates were incubated at 26°C for five days and the fungal flora recorded (Table I). The statistical significance of the data on fungal populations was ascertained by two way analysis of variance<sup>6</sup>.

It was found that populations of *Penicillium*, *Aspergillus*, *Mucor* and *Fusarium* show considerable increase in the rhizosphere of *Macrophomina*-infected jute plants than the healthy ones throughout the experimental period. The populations of *Cunninghamella*, *Verticillium* and *Trichoderma* on the other hand, were found to be considerably decreased in the rhizosphere of *Macrophomina*-infected jute plants than those of healthy ones. *Curvularia* was found to be completely absent in the rhizosphere of diseased jute plants, while in the healthy plants it was found to be present throughout the experimental period. *Spicaria* was found in the rhizosphere soil of healthy plants from the 30th day onwards, but in the rhizosphere soil of infected plants it was totally absent throughout the experimental period. The populations of fungi per gram of soil were found to be higher in the rhizosphere

TABLE I

*The fungal populations in the rhizospheres of diseased and healthy jute plants*

Fungus	Healthy plants				Diseased plants			
	15*	30	45	60	15	30	45	60
<i>Cunninghamella</i> sp.	.. 440†	480	590	550	316	380	290	270
<i>Penicillium</i> sp.	.. 3,020	3,360	4,310	4,750	4,510	5,820	5,450	5,270
<i>Aspergillus</i> sp.	.. 4,420	5,670	5,860	6,270	6,465	7,680	6,837	6,347
<i>Fusarium</i> sp.	.. 410	450	800	950	1,058	1,310	1,214	1,202
<i>Trichoderma</i> sp.	.. ..	740	1,540	2,010	..	..	540	580
<i>Curvularia</i> sp.	.. 610	650	780	960	..	..	..	..
<i>Verticillium</i> sp.	.. ..	450	730	1,050	..	..	360	410
<i>Spicaria</i> sp.	.. ..	308	705	810	..	..	..	..
<i>Mucor</i> sp.	.. 2,145	1,150	1,100	920	3,156	3,380	2,150	1,205

\* Age in days.

† Populations/gram of oven dried sample.

Statistical analysis of the data presented in  
 Table I

Organism	F Value	
	Age effect	Plant effect
<i>Cunninghamella</i> sp.	.. 1.28	14.33†
<i>Fusarium</i> sp.	.. 1.5	39.5†
<i>Penicillium</i> sp.	.. 1.4	36.5†
<i>Aspergillus</i> sp.	.. 1.4	59.2†
<i>Trichoderma</i> sp.	.. 3.9	5.3*
<i>Curvularia</i> sp.	.. 3.05	30.3†
<i>Verticillium</i> sp.	.. 11.4†	7.4*
<i>Spicaria</i> sp.	.. ..	5.9*
<i>Mucor</i> sp.	.. 1.9	8.8*

Table F value at 5% and 1% level = 5.14 and 10.92 respectively for plant condition with df 2 and 6 respectively.

Table F value at 5% and 1% level = 4.76 and 9.78 respectively for age with df 3 and 6.

\* Significant at 5% level.

† Significant at 1% level

soil samples of diseased plants than in the other condition. However, with increase in age of the plant, the populations of different species of the fungi were found to vary considerably, either showing increase or decrease in number. Statistical analysis of the data for age effect shows no significant difference in all the fungal species except in *Verticillium*, where it is significant at 1% level. In case of plant effect significant difference has been obtained at 1% level in case of *Cunninghamella*, *Fusarium*, *Penicillium*, *Aspergillus*, *Curvularia* and 5% level in case of *Trichoderma*, *Verticillium*, *Spicaria* and *Mucor*.

Many workers have shown that disturbed metabolism in the diseased plants results in significant changes in qualitative and quantitative nature of root exudate which possibly accounts for the variation in the rhizosphere mycoflora of the diseased plants from the normal ones. It is interesting to note that in the samples of 15th to 30th day, the populations of most of the rhizosphere fungi in the *Macrophomina*-infected plants were the highest. This may be due to availability of excess of organic matter in the rhizosphere soil as a result of root decay caused by *Macrophomina phaseoli*.

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#### SEED PATTERNS AND GERMINATION BEHAVIOUR IN *CROTALARIA MEDICAGINEA* LAMK. GROWING IN INDIAN ARID ZONE

VARIOUS aspects of seed physiology in arid zone have been dealt by different authors<sup>2,5,7</sup>. The viability and life span of seeds and their state of maturity also have profound effects on seed germination<sup>5</sup>. Among the known method devised for effecting increase in the permeability of hard seed coats, mechanical scarification with concentrated sulphuric acid, boiling water treatment and exposure to suitable temperatures are the ones most commonly followed<sup>1,4</sup>. A clear conception of mechanisms regulating seed germination in Indian arid zone species is to a great extent wanting. Diversity of seeds of different individual plants in a population with regard to their germination behaviour has been reported for some plants in Indian arid zone<sup>3,6-8</sup>. Present investigation deals with germination behaviour of the seeds of *Crotalaria medicaginea*.

While studying the germination behaviour of the seeds of some desert plants, it was found that there exists three types of colour patterns in the seed coat of *C. medicaginea*, i.e., dark black, yellowish-black and yellow. Freshly harvested seeds (last week of September, 1973) did not exhibit any hard seed coat dormancy at the early stage of maturation, but this appeared to develop later when they were fully dried. Such fully dried seeds, neither imbibed nor germinate when provided with suitable conditions. Freshly harvested fully dried seed exhibited an optimum imbibition after a pretreatment with conc. sulphuric acid for 30 minutes. These seeds when stored for a longer period required 45 minutes instead of 30 minutes acid pretreatment for an optimum germination. Seeds taken out from intact fruits, stored for 5 years (collected in 1969) when pretreated with conc. H<sub>2</sub>SO<sub>4</sub> for 45 minutes, showed optimum germination. Such acid pretreatment was tried every month and an optimum germination was always observed with 45 minutes pretreatment. Acid

TABLE I

*Percentage of germination in three types of seeds in C. medicaginea under different pretreatments*

Month of pretreatment	Nature and pretreatment duration	Percentage of germination		
		Black	Yellowish-black	Yellow
October, 1973	.. Conc. H <sub>2</sub> SO <sub>4</sub> —30 mts.	100	100	100
December, 1973 and onward	.. Conc. H <sub>2</sub> SO <sub>4</sub> —45 mts.	100	100	100
February, 1974	.. Dry heat at 70° C—6 days	13	50	..
March, 1974	.. Boiling water—5 mts.	36.0	50	..
April, 1974	.. Imbibed in water—24 hrs.	9.0	17.0	2.0
April, 1974	.. Imbibed in water—24 hrs. (Seeds from intact fruits)	1.0	2.0	1.0

(-) Not tested due to shortage of seeds.

pretreatment for 45 minutes was equally good for all the three types of seeds.

Black and yellowish-black seeds when given dry heat pretreatment at 70° C for 6 days, a higher percentage of germination was observed in the latter, than the former. These two types of seeds did not tolerate a pretreatment of 80° C, but black ones still did not imbibe and germinate even when subjected to such high temperature pretreatment. In the boiling water pretreatment for 5 minutes, yellowish-black seeds imbibed and germinated up to 50% ; while very poor germination was observed in the black ones (Table I). Those seeds which did not imbibe in 5 minutes pretreatment, when given an extra 2 minutes pretreatment, another 50% of these seeds responded.

Approximately five months old seeds of all the three types when kept in water for imbibition, 9% imbibed in black ; 17% in yellowish-black and only 2% in yellow ones (Table I). It has come to light during present experimentation that those seeds which were stored with intact fruits did not imbibe water, irrespective of their seed coat colour. This was even true for the seed collection of 1969. It may be concluded that in intact fruits, the fruit walls prolonged the dormancy of seeds by protecting them from external extreme conditions which made them permeable. To assess that there may be some growth inhibiting substance(s) present in the fruit wall, the seeds were imbibed in the fruit wall extracts, where a certain percentage of seeds did imbibe water. This proves that the fruit wall may be only protecting the seeds from becoming permeable earlier, and thus they are rendered dormant for a longer period.

Further work is in progress. The authors are thankful to the Head, Botany Department, for facilities.

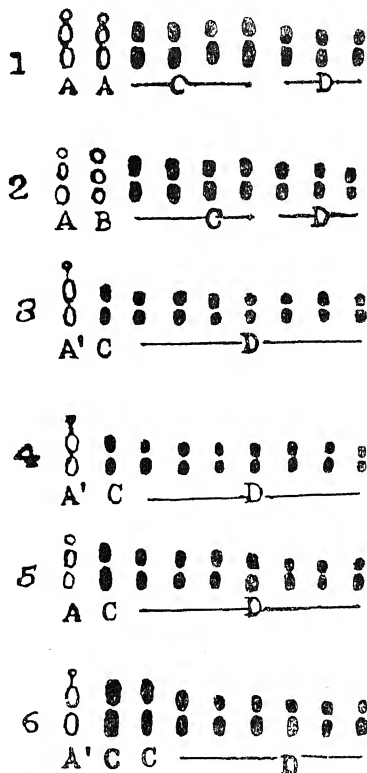
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#### INTERSTRAIN DIFFERENCE IN KARYOTYPE OF *BRASSICA OLERACEA* L.

*Brassica oleracea* L. is Indian Cabbage, cauliflower and knolkhol is popularly cultivated as cole crops throughout India. Cytological studies have been done in this species by Howard<sup>1</sup>, Sikka<sup>2</sup>, Wills<sup>3</sup> and Röbbelen<sup>4</sup>. However, karyomorphological studies on intervarietal and interstrain level have not been done much although such work is needed for proper interpretation of interstrain relationship on structural level. The present paper deals with karyomorphological studies of 6 strains of different varieties

of *B. oleracea* L. with improved technique to study the interstrain difference if any and to trace their evolution through karyotypic study.



FIGS. 1-7. Figs. 1-6. Idiograms of the strains Early Market, Snowball, Sutton's Earliest, Sutton's Express, Drum Head and Earliest White,  $\times 3,000$ . Fig. 7. Photomicrograph of a somatic metaphase of *B. oleracea* var. *capitata* strains, Drum Head with  $2n = 18$  chromosomes.

The materials for the present investigation include two strains of (1) *B. oleracea* var. *botrytis*, viz., (I) Sutton's Snowball (II) Sutton's Early Market; three strains of (2) *B. oleracea* var. *capitata*, viz., (1) Sutton's Earliest (11) Sutton's Express (III) Eclipse Drum Head and one strain of (3) *B. oleracea* var.

*caulocarpa*, viz., Sutton's Earliest White. The seeds were germinated on moist filter-paper in Petridishes and the root-tips were pretreated in Aq. aesculine solution at  $8^{\circ}\text{C}$  to  $10^{\circ}\text{C}$  (Sharma and Sarkar<sup>4</sup>) for  $2\frac{1}{2}$  hours and next Propiono orcein staining technique was followed. Figures were drawn at a table magnification of  $\times 3,000$  using a Zeiss compensating eye piece,  $\times 20$ .

A detailed karyotypic study of six strains shows a similarity in chromosome number ( $2n = 18$ ) and morphology. The length of the chromosome varies from  $3.3\mu$  to  $1.6\mu$ . A critical analysis shows that they differ from one another in minor alterations in the representatives of the types and their different combinations are given in Table I. The description of chromosome types and the varietal karyotype are summarised below.

Type A—Medium sized chromosome with primary and secondary constriction.

Type A'—A medium sized chromosome with primary constriction and satellites.

Type B—Medium sized chromosome, characterised by the secondary constrictions which was located in the middle of the longer arm dividing the chromosome into three more or less equal segment.

Type C—Medium sized chromosome with median to submedian primary constriction.

Type D—Short sized chromosome with median to submedian primary constriction.

The different varieties and strains, their karyotypic configuration and range of chromosome length are given in Table I.

Of six different strains of *B. oleracea* studied here, the chromosome numbers have been observed as  $2n = 18$  confirming the previous report of  $n = 9$  chromosomes in the species (Howard<sup>1</sup>). It is interesting that even though polyploid condition has been observed in this species of *B. oleracea* studied here, yet apparently during cultivation in Indian condition, the diploid has stood the test of selection. The general similarity in chromosome morphology, consisting mainly of medium sized chromosomes which are mostly medianly constricted and with one or two pairs of secondary constrictions, indicates that all the different varieties and strains are allied to each other. However, regarding the basic number in the karyotype, the strains even within a species differ with respect to minute details in chromosome morphology. This fact indicates that even at an intraspecific level minute alteration of chromosomes have been associated in the origin of new strains. Mukherjee<sup>3</sup> also observed such interstrain difference in karyotype in other species of *Brassica*, viz., *B. campestris* L.



TABLE I

Name of the strains	Karyotype	Range of chromosome length
Early Market ..	$2n=18=4A+8C+6D$	$1.8\mu-3\mu$
Snowball ..	$2n=18=2A+2B+8C+6D$	$1.8\mu-3\mu$
Sutton's Earliest ..	$2n=18=2A'+2C+14D$	$1.8\mu-3.3\mu$
Sutton's Express ..	$2n=18=2A'+2C+14D$	$1.6\mu-3.3\mu$
Drum Head ..	$2n=18=2A+2C+14D$	$1.6\mu-2.6\mu$
Earliest White ..	$2n=18=2A'+4C+12D$	$2.4\mu-3.3\mu$

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#### SYNTHESIS OF ACID FROM MANNOSE BY THE SYMBIOTES OF *CLETUS SIGNATUS* WALKER (COREIDAE: HETEROPTERA)\*

ASCORBIC acid is known to be essential for many phytophagous insects (Dadd, 1960). Gilmour (1965) described "it may be that insect tissues in general are incapable of synthesizing ascorbic acid and only those harbouring symbiotic micro-organisms are independent of a dietary supply". Pierre (1962) reported synthesis of ascorbic acid from mannose by the symbiotes of *Leucophaea maderae* (F), whereas the fat body of aposymbiotic insects did not synthesize it. *Cletus signatus* feeds on *Amaranthus* plants. Studies, dealt here, were conducted on the ability of the bacterial symbiotes of this coreid bug to synthesize ascorbic acid from mannose. The ascorbic acid content of the *Amaranthus* inflorescence was also determined and compared with that synthesized by cultured and tissue symbiotes. The effect of kanamycin on the synthesis has also been studied.

**Materials and Methods.**—Total ascorbic acid in the form of dehydroascorbic acid was determined by the combination of the methods of Roe and Oesterling (1944) and Roe and Kuether (1943). Ten mg of cultured symbiotes were scraped from the surface of the nutrient agar slants, homogenized with a glass rod in 2 ml phosphate buffer (pH 7.0) and two ml of mannose solution (0.1 M) were

added in aseptic conditions. The mixture was then incubated for three hours at 30° C in closed tubes. After incubation, 12 ml of 6% trichloroacetic acid and few drops of 2, 6-dichlorobenzene indophenol dye were added. More drops were added until colour persisted, followed by two drops of thiourea solution. The mixture was then centrifuged with chemical centrifuge at 4500 rpm for 20 minutes and 4 ml of supernatant were used for ascorbic acid determinations. Tubes with killed symbiotes were run as checks. Three replications were run for each determination.

For testing the tissue symbiotes, 10 mg of the mycetomal tissue were dissected from adult bugs of *C. signatus*, homogenized by simple grinding with a glass rod in phosphate buffer and synthesis was estimated as described above. Similarly, 10 mg of green inflorescence of *Amaranthus* plant were ground in trichloroacetic acid with a pestle and mortar and ascorbate content was determined. To study the effect of kanamycin on the synthesis of ascorbic acid, the antibiotic was added in one experiment directly into the homogenate (2500 µg/ml homogenate) prepared from 10 mg mycetomal tissue before addition of mannose. In another experiment homogenate was prepared from 10 mg of mycetomal tissue dissected out, 24 hours after injection of kanamycin (600 µg/mg body weight of insect) with a finely drawn glass capillary needle.

**Results and Discussion.**—Results of the experiments summarised in Table I indicate that cultured symbiotes synthesized 1.2 mg of ascorbic acid per gram of bacterial scrappings, while symbiotes in mycetomal tissue synthesized 0.9 mg/gm of tissue in three hours incubation period. When kanamycin was added in homogenate only 0.1 mg of ascorbic acid per gram of tissue was synthesized as compared to 0.85 mg/gm synthesized from the mycetomal tissue of the insects injected with kanamycin. This indicates that the injection of kanamycin in insect body had no effect on symbiotes *vis-a-vis* on ascorbic acid synthesis.

The ascorbic acid content of the inflorescence of host plant was 0.72 mg/gm of tissue. Its relationship with the quantity synthesized by symbiotes

TABLE I

*Ascorbic acid synthesis by the symbiotes of Cletus signatus Walker and ascorbic acid content of Amaranthus viridis inflorescence*

Homogenates of	Amount of ascorbic acid synthesized in 3 hours incubation (mg/gm)
Cultured bacterial symbiotes scrappings	1.2
Mycetomal tissue symboites	0.9
On addition of 1 % kanamycin in homogenate	0.1
Homogenate prepared from the mycetomal tissue of kanamycin treated bugs after 24 hours of injection	0.85
Ascorbic acid content of the <i>Amaranthus</i> inflorescence	0.72

could not be worked out, as neither the ascorbic acid requirements of *C. signatus* were known, nor aposymbiotic bugs be produced. Hence, the importance of such a synthesis by symbiotes to the host insect could not be confirmed in these studies. Pierre (1962) reported same type of synthesis by *Leucophaea* symbiotes and found synthesis of 26.3 µg of ascorbic acid per gram of cultured bacterial scrappings and 33 µg per gram of normal fatty tissue. He reported that aposymbiotic fatty tissues synthesized traces.

It is, however, notable that synthesis is not at all affected by kanamycin injection into the insect body, while the antibiotic, when added directly to the tissue homogenate, reduced it almost totally indicating that the antibiotic was not effective in killing symbiotes, when injected.

The bacterial symbiotes of *C. signatus* synthesized ascorbic acid from mannose (1.2 mg/gm of cultured scrappings and 0.9 mg/gm wet mycetomal tissue). Synthesis was found inhibited completely on addition of 1% kanamycin in homogenate. However, homogenate from kanamycin injected insects synthesized same quantity as from normal insects. Utility of such a synthesis to insects could not be established in present studies due to failure in making insect aposymbiotic.

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# EFFECT OF METHYLBROMIDE

## FUMIGATION ON CHANGES IN NITROGENOUS CONSTITUENTS OF GROUNDNUT SEEDLINGS

METHYLBROMIDE is commonly used for fumigating foodgrains in storage. Therefore, considerable interest has been shown to study the germination of a variety of seeds particularly crop plants fumigated with methylbromide<sup>1-3</sup>. The effect on germination varied with the crop and the initial seed moisture content. It is found<sup>4</sup>, that inhibition of early seedling growth occurred in seeds fumigated with methylbromide. A few physiological studies<sup>5</sup> were carried out on the effect of this fumigant on the metabolic changes associated with the inhibited seedling growth. An irreversible combination of methylbromide with thiol groups in living insects has been demonstrated, especially with the amino acids cysteine and methionine<sup>6</sup>. However so far no such studies have been carried out in plant materials. Therefore the present study is intended to know whether the protein present in the cotyledons is being hydrolysed or not.

The moisture content of groundnut seed (variety TMV<sub>2</sub>) at the time of fumigation was brought to the critical level (15% moisture content on air dry basis). The seeds were fumigated for 24 hr period at the rate of 30 kg per m<sup>3</sup>. Both treated and control seeds were surface sterilized with 0.1% mercuric chloride for one to two minutes, washed with sterile water and germinated on the moist filter-paper in petriplates kept in dark at 23° C ± 2° C. Protein nitrogen was determined separately in the cotyledons and embryonic axis from 1 to 4 days old seedlings. Protein nitrogen and soluble nitrogen fractions were extracted by the method of Thiman and Loos<sup>7</sup> (1947). Nitrogen estimation was done according to Markham<sup>8</sup> (1942) by microkjeldhal distillation.

Soluble nitrogen and insoluble nitrogen (protein) showed a continuous increase in the embryonic axis of the control upto the fourth day after sowing. This shows a continuous degradation of the nitrogen substances in the cotyledons which are translocated into the embryonic axis, while in the embryonic axis of the treated set, soluble nitrogen increased slowly upto the fourth day. But the

TABLE I

*Effect of methylbromide fumigation on changes in nitrogen content (N) in groundnut (mg/seedling)*

Age Days after sowing	Control					
	Cotyledons		Embryonic axis		Seedling	
	Soluble N	Insoluble N	Soluble N	Insoluble N	Soluble N	Insoluble N
0	6.620	14.120	0.040	0.240	6.660	14.450
S.E. $\pm$	0.034	0.518	0.004	0.015	0.126	0.243
1	5.790	12.930	0.080	0.360	5.810	13.290
S.E. $\pm$	0.053	0.253	0.004	0.004	0.134	0.096
2	3.750	11.480	0.150	0.800	3.900	12.280
S.E. $\pm$	0.736	1.235	0.014	0.059	0.016	0.123
3	6.880	8.260	0.240	1.120	7.120	9.380
S.E. $\pm$	0.440	0.307	0.004	0.065	0.152	0.162
4	4.180	9.350	0.540	1.520	4.720	10.870
S.E. $\pm$	0.187	0.437	0.027	0.040	0.003	1.632

TABLE I—Contd.

Age Days after sowing	Treatment					
	Cotyledons		Embryonic axis		Seedling	
	Soluble N	Insoluble N	Soluble N	Insoluble N	Soluble N	Insoluble N
0	6.640	14.220	0.040	0.240	6.510	14.440
S.E. $\pm$	0.351	0.055	0.004	0.006	0.051	0.192
1	5.410	12.960	0.090	0.290	5.600	13.250
S.E. $\pm$	0.093	0.095	0.010	0.002	0.211	0.002
2	4.940	11.360	0.110	0.300	5.110	11.660
S.E. $\pm$	0.574	0.898	0.007	0.004	0.263	0.162
3	4.660	11.300	0.300	0.250	4.960	11.550
S.E. $\pm$	0.208	0.149	0.009	0.003	0.124	0.183
4	4.790	10.660	0.360	0.160	5.150	9.820
S.E. $\pm$	0.039	0.132	0.001	0.002	0.105	0.196

insoluble nitrogen increased only upto the second day while probably parallels with active growth upto that period.

Soluble nitrogen in the treated set decreased upto the second day and remained steady on the following days, while in the control it was fluctuating during two days. Insoluble nitrogen in the cotyledons of the control seedlings gradually went down apparently due to the breakdown of the storage proteins. The insoluble nitrogen in the cotyledons of the treated seedlings decreased only upto the second day from an initial value of

14.22 mg to 11.36 mg (as shown in Table I). Thereafter there was no further decrease up to the fourth day. It obviously indicates that methylbromide prevented the breakdown of the storage proteins. Probably methylbromide might have entered into an irreversible combination of many essential enzymes whose function is known to depend on the integrity of SH enzymes as suggested by Lewis<sup>9</sup>. Therefore methylbromide might be causing inhibition of proteolytic enzymes.

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### CYTOLOGY OF TRIPLOID *ISEILEMA HUBBARDII* MURTY

The smallest chromosome number of  $n=3$  in the Gramineae is found in *Iseilema hubbardii* Murty, a fodder grass belonging to the tribe Andropogoneae<sup>1</sup>. Satyavathi and Murty<sup>2,3</sup> have reported studies on meiosis in this grass and concluded that it might have been derived from another species, *Iseilema antheophoroides* Hack. by repeated reciprocal translocations. They have also reported<sup>4</sup> meiosis in an induced tetraploid of *I. hubbardii*. The tetraploid was crossed with its diploid parent and an autotriploid has been obtained. The present note gives an account of its meiosis.

its diploid parent<sup>2</sup> which is 1.74. The difference was found to be highly significant ( $t=4.21$ ). This decrease is obviously due to the presence of univalents in considerable frequencies.

The trivalents were of 3 types, viz., types 7, 8 and 9 of Darlington<sup>5</sup>, type 7 (chain of three) being the most frequent (Table I). Trivalent type 10 was not encountered. This trivalent requires at least a chiasmata in each of the arms at specific points. This type therefore will be particularly rare in organisms with submedian centromeres and markedly unequal arms<sup>6</sup>. In *I. hubbardii*, all the 3 chromosomes are submedian<sup>2</sup> which explains the non-occurrence of trivalent type 10.

TABLE I

Frequency of chromosome associations in triploid *Iseilema hubbardii*

Sl. No.	Frequency of chromosome associations							No. of cells observed
	I	II	III					
	I	( )	I	Y	( )	(I)		
1.	3	1	2	0	0	0	0	12
2.	3	1	2	0	0	0	0	11
3.	1	1	0	2	0	0	0	7
4.	0	0	0	1	1	1	0	12
5.	0	0	0	2	1	0	0	9
6.	0	0	0	1	0	2	0	9
TOTAL	76	30	46	53	21	30	0	60

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FIGS. 1-2. Meiosis in triploid *Iseilema hubbardii* ( $n=3$ ). Fig. 1. Diakinesis showing 3 I, 3 II. Fig. 2. Diakinesis showing 3 III.

The triploid was highly sterile with a pollen fertility of 10.6%. Meiosis was irregular with the formation of univalents and trivalents. The frequencies of univalents, bivalents and trivalents were 1.27, 1.27 and 1.73 respectively. The number of half chiasmata per chromosome was 1.33 at diakinesis. This is less than that of

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## RAMGARH METEORITE CRATER

THE authors confirm the discovery of a meteorite-impact crater near Ramgarh, a small village in Kota District, Rajasthan. A map and an aerial photograph of this structure had been published in the *Survey of India School Atlas*<sup>1</sup>, and these maps were used to make a detailed study of the astrobleme. Crawford<sup>2</sup> (1972) has expressed his opinion that it may be a meteorite impact structure.

During the course of this study the authors collected few highly magnetic pieces with characteristic pitting and polish from inside the crater and magnetic spherules from the clay outside the impact area. A preliminary study in the Laboratories of the Geology Department, Aligarh Muslim University, Aligarh, has revealed that these pieces could have been a part of nickel-iron meteorite.

*Geology and the structure of the crater.*—Along the inner side of the eastern wall, just near the entrance of the crater, the following geological succession was recorded :

Red Quartzitic sandstone (Bhander sandstone)  
about 180 m

Arenaceous Limestone (Bhander) about 5 m

Red shale (Bhander) about 5 m

Green shale (Bhander) about 10 m.

Entrance to the crater is along a hinge fault, running south-west to north-east. The eastern wall of the entrance being the upthrow side, exposes the limestone and shales. These beds are forming a monocline, dipping towards the south-east at an angle of 32°. Almost in the middle of the crater and below the ruined temple, green shales are exposed dipping vertically, probably due to the impact of the meteorite<sup>3</sup> (1971). No other exposure of the bed rock was seen on the crater floor but it may be concealed under the lake bed. The crater walls are made up of Bhander sandstone, dipping radially outwards, at angles between 32° and 54°. At one point along the crest of the western wall the dip is as high as 75° and on the crest of the northern wall the beds are dipping towards the interior at angles of 20°–35°.

Along the western outer margin of the crater the beds are dipping towards the north and east (outwards) at angle greater than 18°, but on the eastern side the beds are almost horizontal and the dips are not higher than 3°. All around this crater, the angle of dip decreases down hill from the crest and even a few yards away from the outer rim of the crater, the dips are invariably nominal and do not exceed 2°–3°. The rocks along the crest of the crater wall are highly fractured but the intensity of fracturing also decreases down hill.

The hinge fault near the entrance, mentioned above, is obviously responsible for stream draining the crater. The lakes inside the crater are also oriented along the axis of this fault. This fault has also possibly affected the crater wall on the north-east but no displacement was noted in this area, and from the available evidence it appears that it dies out in the middle of the crater.

*Origin of the crater.*—An examination of the crater convinced the authors that it was not of volcanic origin. At the same time, it did not show any evidence of igneous intrusion. For had there been an igneous intrusion, which cooled and allowed the bubble to collapse, the fracture pattern would have been more or less symmetrical and significantly different from what is seen in the area. Similarly, it was not a sub-surface salt dome from which salt was subsequently dissolved by percolating water, and the whole edifice collapsed thereafter.

In the absence of any other evidence and on the basis of the two magnetic pieces of nickel-iron found in the crater and magnetic spherules collected from outside the crater area, the authors are of the opinion that the crater was formed due to the impact of a meteorite. It need hardly be pointed out that no magnetite exists in the country rocks for scores of Kilometres around. The shape of the crater, and the fact that nowhere from the Vindhyan plateau any igneous activity is reported confirms this view. Indeed, nowhere the Vindhyan beds are even moderately disturbed, except where normal faults are present.

Measured from the top of the rim on one side to the top of the rim on the other the crater has a diameter of more than 5500 metres.

A detailed study of the crater and the meteorite pieces are in hand. The authors are grateful to Prof. F. Ahmad, Head of the Geology Department, Aligarh Muslim University, Aligarh, for his valuable suggestion in the field as well as in the laboratory. They are also thankful to Mr. M. Ishaq and Mr. M. Ashfaq, Advocates Baran and Mr. Mohan, Secretary, Ramgarh Panchayat, for providing accommodation and other facilities in the field.

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H. A. SAJJID.  
IQBAL HASNAIN.

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## REVIEWS AND NOTICES OF BOOKS

Modern Organic Chemistry. By S. P. Pathak and P. B. Mahajani. (Dastane Ramachandra and Co., Poona), 1974. Pp. 588. Price Rs. 28-00.

This book is made up of 31 chapters, besides two appendices containing problems and questions and an index and a brief bibliography (reference to 17 books).

The first 230 pages cover reaction mechanisms, stereochemistry, spectroscopy and some related topics. About 40 pages are devoted to biochemical topics which include proteins, enzymes, biochemical genetics and 'chemistry of life'. In so far as the topics are important, many of them modern and worthy and necessary to be taught to students, the authors have attempted to bring this book in step, with modern foreign texts. The rest of the 300 odd pages cover conventional topics.

In a text book of this sort, what should be included, what should be emphasized, and what should be left out, would always remain a question of personal judgement. Even so, some omissions seem conspicuous: pericyclic reactions, nonbenzenoid aromatics, biosynthesis, photochemistry, chemistry of arynes, Wittig reaction, 1, 3-dipolar additions. Steroids are dismissed of in a page and half, Diels-Alder reaction, gets a cursory treatment without its stereochemical implications.

To claim to describe "factors controlling reaction mechanism" (Chapter 6), without even a mention of thermodynamic considerations and the concept of electronegativity, is unrealistic.

One of the more serious shortcomings of the book is its inadequate, generally imprecise, and often unrestrained expression.

Consider the following statements:

Page 1:

"With the help of a few fundamental principles, we can predict how an organic compound should behave, which type of reactions it should undergo, and with what speed."

E. Bright Wilson Jr.<sup>1</sup> writing in "Some Remarks on Quantum Chemistry" gives a different picture:

"Quantum mechanics has not yet attained the status of thermodynamics in chemistry. Despite forty years of effort by thousands of investigators, it is largely an article of faith that the Schrödinger equation is capable of explaining all the facts of chemistry" (p. 753).

"Despite past and prospective progress in pure theory, I fear that chemistry will continue to be largely empirical. There is no disgrace in

empiricism, and no need or advantage in disguising it as pseudo theory" (p. 759).

Compare the following statement in p. 210, para 2 of the text book,

"Infra-red spectroscopy is the best tool for organic chemistry, for investigation of structure of unknown compounds,"

with that of D.H.R. Barton<sup>2</sup>, "nuclear magnetic resonance is the single most important physical tool available to the organic chemists".

The major weakness of the book, however, arises from needless presentation of unsupported personal viewpoints, and its misleading representation of facts and concepts.

The following statements are illustrative.

Page 204, para 3:

"Even though energy point of view (sic),  $n$  to  $\pi^*$  transition appears to be the easiest, it is geometrically poor, since  $n$  and  $\pi^*$  orbitals are at an angle with each other. These  $n$  to  $\pi^*$  absorptions have low intensity and, therefore, not taken seriously."

This transition, happens to be the basis of the well known Octant Rule and its study was the starting point for the renaissance of interest in Optical Rotatory Dispersion and Circular Dichroism, which led to their adoption as practical tools in organic chemistry (ref. 3).

On p. 58 of the text book the following statement is made:

"Molecular orbitals, i.e., chemical bonds." For a different view see M. Orchin and H. H. Jaffe<sup>4</sup>—  
"The importance of antibonding orbitals."

Page 58, para 2:

"Bond energies and the corresponding bond dissociation energies are numerically identical, but have opposite signs." Refer for a different account see L. Pauling<sup>5</sup>—"The Nature of the chemical bond".

Page 6:

"The difference in the internal energy of the boat form and the chair form is only 6 kcal/mole. Hence even at room temperature, these two forms of cyclohexane are easily interconvertible and can be called conformations of cyclohexane."

The authors apparently do not see the difference between *barrier to rotation* and *energy difference* between isomers.

These serious mistakes are not few, or far between, as to be dismissed as inevitable in a text book. The reviewer has come across over fifty without looking for them (not counting trivial

errors). In fact, discussions of theoretical principles not vitiated by improper or inaccurate presentation are few.

It is not the purpose of the reviewer to suggest that these authors have done worse than some of the text book writers. They have only broken with tradition, to venture and cross the safe shores, with inadequate preparation.

M. V. BHATT.

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**Interactive Computing in Basic.** By Peter C. Sanderson. (Butterworth Group, 88 Kingsway, London, WC. 2B, 6AB), 1973. Pp. vii + 161. Price £4.00.

This book gives the general introduction to the use of digital computer *via* remote terminals using the language BASIC (Beginners All-purpose Symbolic Instruction Code) first implemented in 1965. This facility of having a number of remote terminals attached to the computer for the use by equal number different users simultaneously, without anyway disturbing each other or the working in CPU room, gives the feeling to every terminal user that he is the only user of the computer system. At present this facility is rarely available in India. Such a use of the computer is known as interactive use as the computer interacts with the terminal user, by responding to the input information key-punched by the user, by way of giving instant error diagnosis and thus giving the user immediate opportunity of correcting the invalid input. Again, the facility of supplying data during execution of the program, providing interaction between user and the computer, is available. This book mainly teaches the

language BASIC which is convenient for interactive use. Some information about the organisation of an interactive system is included.

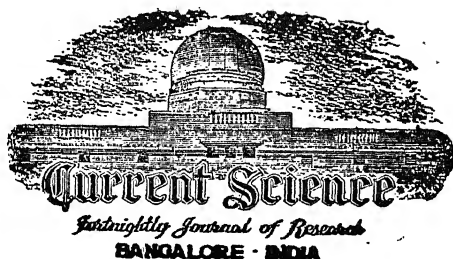
Some of the improvements suggested are the following: (1) Page 66: The number 1170 in the statement giving the form of RESTORE statement should be dropped as is done for GO TO statement on page 70. (2) As the discussion comes after that of STOP statement, "uses to a statement" be replaced by "uses to a IF statement" in line 6 from the bottom on page 75. (3) In the first program on page 81, the statement number 1010 should be INPUT Z as READ statement can never appear without a corresponding DATA statement. (4) It will be better if what happens when an expression after ON turns out to have a non-zero fractional part is stated before the program utilizing ON GO TO technique is presented on page 81. (5) It is better to call M as 'test value' rather than 'terminal value' in FOR statement on page 85 in view of its function stated in the beginning of page 86. (6) To use word 'expression' in place of 'value' in line 14 on page 87 would add to clarity. (7) What action compiler will take if the initialisation of the value of J is bypassed, as in first program on page 88, need be stated. (8) Period symbol (.) should appear at the end of the output shown in line 17 on page 111. Statement 460 of the program on page 111 should contain ',' instead of ';' between N and Y. (9) There should be a READ statement in the program corresponding to the DATA statement 560 on page 113. (10) On page 130, J should be replaced by M in second line after first illustration of calling a subroutine, and LEQ.O should be included in the brackets in the left hand program at the bottom of the page. (11) Formula in problem 3 on page 69 is incorrect. The corresponding program written on page 143 is for the correct version of the formula.

The language of the book is simple and informative, but insipid. '740' in place of 74 in 6th line on page 76 is a printer's devil. Exercises for practice (with suggested solutions at the end) are spread throughout the book. Variants of BASIC and some simple rules for the conversion of BASIC programs to FORTRAN are included.

V. G. TIKEKAR.

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# $^{18}\text{O}/^{16}\text{O}$ AND $^{13}\text{C}/^{12}\text{C}$ RATIO VARIATIONS IN KAJARHAT LIMESTONES

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AND

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## ABSTRACT

Oxygen-18 and carbon-13 isotopic analyses of Kajarhat limestones from Bhawanathpur area of Palaman District have been carried out. The isotopic data for both carbon and oxygen are in the range of exchanged marine carbonates of Cambrian age. The irregular correlations between the  $\delta$  values of the samples from the area have been attributed to different extent of dolomitization and post-depositional exchanges with fresh water and carbon dioxide.

## INTRODUCTION

THE study of oxygen-18 and carbon-13 isotope distribution in limestones and carbonates have been used for the elucidation of the environmental conditions of formation (Sharma and Sharma, 1970; Pandey *et al.*, 1969; Pillai and Sharma, 1969; Singh *et al.* 1973). Usually the isotopic composition of limestones are dependent upon the temperature of deposition, source of formation and the postdepositional alterations. Limestones are usually deposited in the sedimentary environments at low temperature and their  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values are higher compared to metamorphic and igneous rocks and minerals as suggested by Silverman (1951). The deposition of limestones in marine environment gives higher  $\delta^{18}\text{O}$  compared to fresh water carbonates due to higher  $\delta^{18}\text{O}$  value for marine water. The isotopic exchange of the carbonates of the limestone during and after the deposition with fresh water and atmospheric or biogenic carbon dioxide alters the original  $\delta$  values of the limestones.

In the present work several limestone samples from different sections of Bhawanathpur area (latitude  $24^{\circ} 25'$ , longitude  $83^{\circ} 35'-36'$  approx.) in the District of Palamau, Bihar, have been studied. The sample belongs to lower Vindhyan Kajarhat limestones of the Basal stage of the Semri series. The isotopic analyses of the samples have been carried out with a view to ascertain the extent of alteration and isotopic exchange.

## EXPERIMENTAL

Carbon dioxide was extracted from the samples using 100% phosphoric acid (McCrea, 1950) according to the procedure suggested by Sharma and Sharma (1969). The mass spectrometric analyses were carried out on a 6"-60"-RMS-19 double collecting isotope ratio mass spectrometer

in the Department of Chemistry, I.I.T., Kanpur. The isotopic data are reported in terms of  $\delta$  terminology defined as :

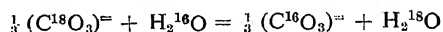
$$\delta_{\text{‰}} = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

where  $R = ^{18}\text{O}/^{16}\text{O}$  or  $^{13}\text{C}/^{12}\text{C}$  ratio. The  $\delta$  values are corrected for instrumental effects (Craig, 1967) and are given in terms of SMOW and PDB for oxygen and carbon respectively.

The reaction of the limestones with phosphoric acid was found to be slow indicating the presence of dolomite. X-ray analysis of the samples were carried out on a G.E.C. X-ray diffractometer using a Cu-target and  $d(112)$  reflections were recorded by scanning the chart between  $28.5^{\circ}$  and  $32^{\circ}$ . The samples showed the calcite and dolomite characteristic peaks around  $29.5^{\circ}$  and  $31^{\circ}$  respectively.

## RESULTS AND DISCUSSION

The peak heights of the samples from the X-ray spectrum have been shown in Table I. The isotopic data of the limestones are shown in Table II. The  $\delta^{18}\text{O}$  values of the limestone are lower than the average unaltered limestones (28 to 32‰). The lowering of the  $\delta^{18}\text{O}$  is attributed to exchange with fresh water according to the reaction



Epstein *et al* (1964) have demonstrated that  $\delta^{18}\text{O}$  values of limestones change with age due to exchange with fresh water. As the samples belong to lower Vindhyan, the lowering of  $\delta^{18}\text{O}$  due to exchange with fresh water, is plausible.

The Z values calculated for the samples under investigation according to the equation of Keith and Waber (1964) are listed in Table III. The values are around 120 and thus are indicative of exchanged marine nature of the samples as

TABLE I  
X-Ray data for limestone samples

Sample	Calcite peak	Dolomite peak	Ratio Cal./Dol.
A <sub>8</sub>	35.0	7.5	4.66
B <sub>1</sub>	59.5	20.0	2.43
B <sub>2</sub>	21.5	7.0	3.71
B <sub>3</sub>	8.0	23.5	0.35
B <sub>4</sub>	3.5	53.5	0.06
B <sub>5</sub>	11.0	43.0	0.28
C <sub>1</sub>	35.5	5.25	6.17
C <sub>2</sub>	33.0	14.0	2.35
C <sub>3</sub>	32.5	27.5	1.18
C <sub>4</sub>	25.0	20.5	1.22

TABLE II  
Isotopic data for limestones

Sample	$\delta^{18}\text{O}$ (SMOW)	$\delta^{13}\text{C}$ (PDB)
A <sub>8</sub>	19.42±0.06	-0.62±0.05
B <sub>1</sub>	17.62±0.14	-1.36±0.04
B <sub>2</sub>	21.08±0.06	-1.32±0.12
B <sub>3</sub>	21.14±0.05	-1.77±0.08
B <sub>4</sub>	21.46±0.13	-0.23±0.06
B <sub>5</sub>	22.97±0.06	-0.32±0.02
C <sub>1</sub>	17.92±0.06	-1.04±0.05
C <sub>2</sub>	19.62±0.17	-0.86±0.14
C <sub>3</sub>	23.44±0.17	-0.52±0.16
C <sub>4</sub>	21.06±0.11	-10.18±0.14

± Represents average deviation for three analyses.

TABLE III  
Z values of the limestone samples

Sample	$\delta^{18}\text{O}$ (PDB)*	$\delta^{13}\text{C}$ (PDB)	Z†
A <sub>8</sub>	-9.78	-0.62	121.1
B <sub>1</sub>	-11.58	-1.36	118.7
B <sub>2</sub>	-8.12	-1.32	120.6
B <sub>3</sub>	-8.06	-1.77	119.6
B <sub>4</sub>	-7.74	-0.23	123.0
B <sub>5</sub>	-6.23	-0.32	123.5
C <sub>1</sub>	-11.28	-1.04	120.0
C <sub>2</sub>	-9.58	-0.86	120.8
C <sub>3</sub>	-8.14	-1.18	120.8
C <sub>4</sub>	-5.76	-0.52	123.4

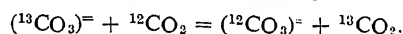
 $\delta^{18}\text{O}$  (PDB)\* =  $\delta^{18}\text{O}$  (SMOW) - 29.2‰.Z† = 2.048 ( $\delta^{13}\text{C} + 50$ ) + 0.498 ( $\delta^{18}\text{O} + 50$ ).

suggested in our previous communication (Singh *et al.*, 1973).

The variation of  $\delta^{18}\text{O}$  values may be explained on the basis of the extent of alteration, i.e., dolomitisation of the limestones. Different peak heights of the samples indicate different amount of dolo-

mite and consequently different extent of dolomitisation. Calcite (calcium carbonate) exchanges faster with water as compared to dolomite and hence samples containing more dolomite should be heavier in  $^{18}\text{O}$ . From the peak heights, ratio of calcite and dolomite and  $\delta^{18}\text{O}$  values no correlation is observed with the dolomite content. The absence of correlation may be attributed to the mixed processes operative in dolomitisation. If dolomitisation occurs by metasomatic cation exchange process there should be no difference in the  $\delta^{18}\text{O}$  values for calcite and dolomite. Whereas in the event of formation of dolomite from the dissolution of calcite by solution process it should enrich the dolomite content by 4 to 6‰. Thus the correlation between  $\delta^{18}\text{O}$  and dolomite content is likely to be lost due to the formation of dolomite by a combination of the metasomatic and the hydrothermal processes.

There is no regular correlation between  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values and some of the samples show very low value of  $\delta^{13}\text{C}$  which may be attributed to the postdepositional exchange of the carbonate with biogenic carbon dioxide according to the equation :



This type of exchange lowers the  $\delta^{13}\text{C}$  whereas the  $\delta^{18}\text{O}$  value is not appreciably affected.

## ACKNOWLEDGEMENT

We are thankful to Professor V. N. Singh, Department of Geology, Patna University, for the samples and the geological informations.

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## AFATOXIN PRODUCTION IN SUNFLOWER (*HELIANTHUS ANNUUS*) SEED VARIETIES

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### ABSTRACT

Sunflower seeds have been for the first time shown to support aflatoxin production. The toxin production in five varieties of sunflower seeds has been studied. Under laboratory conditions, considerable variation in toxin production was seen among the five varieties. The possible causes for the low toxin production on whole seeds have been examined. The hard seed coat of the whole seeds impedes the penetrability of the fungus thus resulting in low toxin production.

**A**FLATOXIN contamination has been encountered in several agricultural commodities and more so in oil seeds<sup>1</sup>. Extensive research has been in progress on several aspects of aflatoxin problem in peanuts and to some extent in soyabeans and cotton seeds. However, this problem has not been adequately investigated on other oil seeds. Aflatoxin production on sunflower seeds (*Helianthus annuus*) has so far not been studied. It is perhaps important to investigate this as well since sunflower seed holds the second place in international oil production and is the fourth largest source of oil seed protein in the world<sup>2,3</sup>. Russia had been the largest producer of sunflower seeds so far, though efforts are currently being made to cultivate this oil-seed crop extensively in U.S.A.<sup>4</sup> and other regions of the world. In India, there has been a crash programme to boost the production of several oil-seed crops including sunflower seeds. Current estimates indicate that at least 3,50,000 hectares are under sunflower seed cultivation in the southern regions of the country<sup>5,6</sup>. Sunflower seed is known to possess certain unique advantages over soyabeans since its oil yield is greater and its meal is practically devoid of any toxic material and will therefore be useful as protein-rich feed for poultry and human consumption as well. In fact preparations made from deoiled meal have been successfully used as protein-rich supplements to pre-school children<sup>2</sup>. In view of the importance of sunflower seed as source of oil and protein-rich meal, it was of interest to investigate the aflatoxin production on some promising varieties currently released for extensive cultivation. The present communication deals with the toxin production on five varieties of sunflower seeds.

### EXPERIMENTAL SECTION

**Material: Sunflower seeds.**—Five authentic varieties of sunflower seeds (EC. 68413, EC. 68414, EC. 68415, EC. 69874 and sunrise selection) were obtained from Oil Seed Specialist, Andhra Pradesh Agricultural University, Hyderabad,

**Fungal isolates.**—Two isolates of *Aspergillus flavus* (NIN. 169 and NIN. 195) and three isolates of *Aspergillus parasiticus* (NRRL. 2999, RIB. 4002 and NIN. 204) were used.

**Methods.**—Twenty gram lots of each variety of sunflower seeds (whole seed or broken seeds) were rehydrated with 10 ml of water, sterilised by autoclaving at 15 lb pressure per sq. in. for 15 min. The flasks were then inoculated with a uniform spore suspension of the fungal isolates and incubated at 28° C for seven days. At the end of the incubation period, the samples were sprayed with 95% alcohol and dried overnight at 80° C. The dried samples were first defatted with *n*-hexane and then extracted with methanol. The aqueous methanolic extracts were treated with 20% lead acetate to remove interfering pigments. The filtrates after lead acetate treatment were extracted with chloroform. The chloroform extracts were concentrated and used for thin layer chromatography using chloroform : methanol (95 : 5) as the developing solvent system. The aflatoxin B<sub>1</sub> content was quantitated by the method described by Pons *et al.*<sup>7</sup> using a pure reference standard toxin B<sub>1</sub>.

### RESULTS AND DISCUSSION

The aflatoxin B<sub>1</sub> production by different fungal isolates on five varieties of sunflower seeds are indicated in Table I. The toxin production was usually related to the toxigenic potential of the isolates of *A. flavus* and *A. parasiticus*. The varieties also exhibited variation in toxin producing capacity.

A comparative study of the toxin production potential of three categories of oil seeds are presented in Table II. Using the same strain (NRRL. 2999), the toxin production on peanuts and soyabeans have been examined in earlier studies<sup>8,9</sup>. This indicates that the aflatoxin producing capacity of sunflower seeds is markedly lower than peanuts and soyabeans.

An attempt was, therefore, made to examine why sunflower seeds support only minimum toxin elaboration even under optimal conditions. In this study

TABLE I  
Aflatoxin production on varieties of sunflower seeds

Isolate	Toxin (B <sub>1</sub> ) produced in parts per million				
	EC. 68413	EC. 68414	EC. 68415	EC. 69874	Sunrise selection
NIN. 169, <i>A. flavus</i>	.. 0.62	0.62	0.80	1.56	1.56
NIN. 195, "	.. Nil	Nil	< 0.1	< 0.1	< 0.1
NRRL. 2999, <i>A. parasiticus</i>	.. 15.6	6.13	15.6	15.6	6.13
NIN. 204, "	.. < 0.6	< 0.1	< 0.3	< 0.1	< 0.1
RIB. 4002, "	.. 3.12	3.12	0.06	0.06	0.25

TABLE II  
Aflatoxin production on oil seeds

Oil seed	Protein %	Oil %	Range of aflatoxin yield in ppm
Soyabean	.. 40-45	20-28	20-30
Sunflower	.. 18-21	45-50	6-16
Groundnut	.. 20-25	47-49	25-50

four varieties of sunflower seeds were used. It was thought that thick seed coat in sunflower seeds might be responsible for the low toxin production. In a fresh series, whole seeds and broken seeds of the four varieties were again infected under identical conditions as described. The toxin production under these conditions is given in Table III. These data appear to suggest strongly that the "armoured seed coat"<sup>10</sup> perhaps interferes with the penetration of the invading fungus and subsequent ability to produce the toxin. This is indicated by the fact that toxin production in broken seeds is markedly higher than on the whole seeds and is almost comparable as on other oil seeds such as peanuts and soyabeans.

TABLE III  
Aflatoxin production on whole and broken seeds of different varieties of sunflower

Sunflower varieties	Aflatoxin B <sub>1</sub> produced* in parts per million	
	Whole seed	Broken seed
EC. 68413	.. 15.62	39.05
EC. 68414	.. 6.25	39.05
EC. 68415	.. 15.62	39.05
Sunrise selection	.. 6.25	15.62

\* Isolate NRRL. 2999.

These observations suggest that aflatoxin contamination can be encountered in sunflower seeds and accumulation of the toxin could be considerably minimised by storing the sunflower

seeds with the seed coat and could be dehulled just prior to extraction of oil. The hardy nature of the seed coat offering resistance to the penetration of fungi is perhaps not an uncommon phenomenon. Such instances have been observed in certain varieties of peanuts<sup>11</sup> and cotton seed varieties<sup>12</sup>.

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## BRUCITE MARBLES OF DEVGAD BARIA AREA, PANCHMAHALS DISTRICT, GUJARAT

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### ABSTRACT

Geological investigations of the area around Devgad Baria have revealed the occurrence of brucite marbles in proximity to the graphite bands in the Banded Gneissic Complex. The detailed field and petrographic studies have shown that brucite is developed from the dolomite in a single reaction during the contact metamorphism and the associated graphite is a result of this reaction formed from the expelled carbon.

### INTRODUCTION

IN the course of detailed geological investigations of the area around Devgad Baria ( $22^{\circ} 42' : 73^{\circ} 54' 30''$ ) brucite marbles have been encountered in the Banded Gneissic Complex. The purview of literature on the area indicates that there is no detailed account of the brucite marbles except for the passing reference made by Sahu and Mungee<sup>1</sup>. The detailed geological studies on the then Baria State by Rama Rao<sup>2</sup> did not record the brucite marbles.

The various rock types of the Banded Gneissic Complex include biotite-schists and -gneisses, arkoses, calc-silicate rocks, marbles, graphite-schists and -gneisses and amphibolites striking east-west in general with local variations to WNW-ENE and dip towards north at angles ranging from  $40^{\circ}$  to  $70^{\circ}$ . They are banded intricately and the bands show variation in thickness, colour and mineral assemblage, and are intruded by granitic veins.

The marbles crop out as disconnected lenses at many places, but the brucite marbles are found in the vicinity of graphite bands near Sewania ( $22^{\circ} 35' : 73^{\circ} 59'$ ) and to the north of Nadatod ( $22^{\circ} 36' : 73^{\circ} 57'$ ) villages. They exhibit different colours in shades of green, greyish blue and white and show gradual variation through calc-silicate rocks to the arkoses.

### PETROGRAPHY

The *brucite marbles* are coarsely crystalline containing dull yellowish white crystals of brucite amidst the pale grey to bluish grey crystals of dolomite and calcite. They have a specific gravity of 2.63. Small flakes of mica showing yellowish brown colour are also common in the rocks. A rude banding of the rocks seems to have been disturbed by the formation of brucite.

In thin section, the rocks show granoblastic texture and comprise brucite, calcite, dolomite, phlogopite, clinocllore, graphite, diopside and wollastonite. Modally brucite forms 36% of the

rock, calcite and dolomite combined together ranges upto 57% and the rest is in accessory amounts.

*Brucite* which has been confirmed by staining tests (Heinrich, p. 13)<sup>3</sup>, is colourless, rounded in form and is fibrous with roughly concentric onion-like (whorls) shells. The layers are made up of tiny concave scales of fibrous nature. The fibres lie at right angles to the scale surfaces and so radial to the layers. Some are, however, at an angle of  $45^{\circ}$  to this direction. In a few cases, the fibres in adjacent scales are inclined in opposite directions, giving a herringbone pattern. There is no relict periclase inside brucite, but some calcite grains are enclosed in the mineral. Thin veins of calcite cut across the brucite and the enclosed calcite and a few graphite flakes are found in association with brucite. *Calcite* shows typical rhombohedral cleavage and characteristic lamellar twinning, with their twin lamellae often bent. *Dolomite* is identified by the presence of twin lamellae parallel to the short diagonal, and in some cases to both long and short diagonals of the cleavage rhomb thus forming rectangular grid, and by simpler forms. *Phlogopite* occurs in flaky form; pleochroic from colourless to yellowish brown; and  $Z\Delta a$  is  $5^{\circ}$ . Some crystals are bent indicating the effect of shearing stress. *Clinocllore* is colourless and gives straight extinction. The interference colour is grey of first order. The crystals are bent,  $2V$  is  $(+)$   $30^{\circ}$  and the mineral is often oblique to the alignment of phlogopite flakes probably indicating its formation later than the phlogopite (Harry)<sup>4</sup>. *Graphite* is flaky and has crinkled margins. It is disseminated throughout the section, but is abundant in the bluish grey band associated with brucite. *Diopside* is subhedral to euhedral in outline; it is colourless; cleavage traces parallel to (100) are prominent;  $Z\Delta c$  is  $44^{\circ}$ ; the maximum interference colour is blue of second order and  $2V$  is  $(+)$   $57^{\circ}$ . *Wollastonite* is bladed in form, slightly lower in relief than

the diopside and displays yellow and grey of first order interference colours.  $Z\Delta c$  is  $30^\circ$ . The minerals diopside and wollastonite occur in the form of small veinlets cutting across the brucite marble.

#### METAMORPHISM

The mineral assemblage of calcite, dolomite, and phlogopite in the present rocks and the occurrence of amphibolites and forsterite marbles along with the directed textures in the Complex indicate that the rocks were initially metamorphosed to amphibolite facies (Turner)<sup>5</sup>.

Later the intrusion of granitic rocks has brought about contact metamorphism in the country rocks (Narayana)<sup>6</sup>. The development of brucite and diopside suggest relatively high temperature and low pressure conditions of metamorphism diagnostic of hornblende-hornfels facies (Chaudhuri)<sup>7</sup>. Wollastonite is noticed in the form of minute veins in brucite marble, and it is taken as an indication of pyroxene-hornfels facies developed in proximity to the intrusions in the area following Chaudhuri<sup>7</sup>. This conclusion gets support from the mineral assemblages of the xenoliths (amphibolites, phylites and quartzites) found in the granitic rocks of the area (Narayana)<sup>8</sup>.

Thus the marbles along with the other components of the Banded Gneissic Complex of the area have suffered amphibolite facies of regional metamorphism followed by hornblende-hornfels facies of thermal metamorphism which attained pyroxene-hornfels facies close to the granitic intrusions.

#### FORMATION OF BRUCITE

There are three ways in which brucite can originate in calcareous rocks undergoing contact metamorphism:

- (a) alteration of serpentine,
- (b) de-carbonation of magnesian limestone to form periclase, which subsequently gets hydrated to form brucite, and
- (c) de-carbonation of dolomitic limestone with accompanying hydration and without the formation of periclase as an intermediate member.

The brucite in the marbles of the present area shows no evidence of its formation by alteration of serpentine. Rounded grains of serpentine occur in some of the marbles which do not contain

brucite and, moreover, the serpentine often forms along the margins and cracks of forsterite. Further, the absence of any residual periclase in the brucite casts doubt on the applicability of the second mode of formation mentioned above to the genesis of brucite.

On the other hand, presence of calcite inside brucite and association of graphite with brucite are suggestive of the mineral being formed directly at the expense of dolomite by the elimination of  $\text{CO}_2$  and substitution of  $\text{H}_2\text{O}$  in a single reaction of the metasomatic process. Lemberg (1874, referred to by Brown)<sup>9</sup> proved the change of dolomite to a mixture of calcite and brucite experimentally under similar conditions. Brown<sup>9</sup> and Smolin<sup>10</sup> have also described brucite formed in this way. Thus the vein-like calcite cutting the brucite and the calcite enclosed in brucite may represent the released product during the formation of brucite from dolomite and the association of graphite flakes with brucite may also suggest, in a similar way, their formation from the expelled carbon.

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# BIOTITES FROM THE GRANITIC ROCKS AROUND DORANDA IN THE EASTERN PART OF THE BIHAR MICA BELT

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## INTRODUCTION

**T**HE eastern part of the Bihar Mica Belt around Doranda (24° 28' N; 85° 27' E) in the District of Hazaribagh, consists mainly of granite gneisses, quartzite, hornblende schist and mica schist. The granite gneisses are highly feldspathised with the development of K-feldspar megacrysts in the eastern and western parts of area.

Detailed structural and petrographic studies (Ghosh, 1971) indicate that the granite gneisses are products of metasomatic transformation of pre-existing metamorphites, chiefly mica schist and hornblende schist, while the K-feldspar megacrysts developed due to a later potash metasomatism.

The refractive indices of biotite ( $N_z$ ) for thirty-one samples (Table I) of these granite gneisses have been determined by liquid immersion method using sodium light; precision of the determination is  $\pm 0.002$ . The values of  $N_z$  vary within wide limits (1.624 and 1.662). Three samples of biotite with varying refractive indices were separated by means of a magnetic separator, tapping on glazed paper and finally by hand picking under the microscope for partial chemical analysis (Table II). The final purity of the sample was about 99%.

TABLE I

Refractive index of biotite from thirty-one samples of Doranda granite-gneiss

Sp. No.	$N_z$	Sp. No.	$N_z$
1	1.625	400	1.628
291	1.639	63	1.628
15	1.620	402	1.632
2	1.634	60	1.622
30	1.624	246	1.625
55A	1.628	245	1.623
40	1.625	403	1.628
196	1.625	391	1.624
41	1.624	392	1.628
47	1.632	443	1.634
36	1.625	404	1.628
5A	1.623	79	1.662
64	1.628	78	1.642
66	1.625	425	1.625
201	1.625	200	1.628
		401	1.629

TABLE II

Partial chemical analyses and  $N_z$  of biotite in three specimens of Doranda granite-gneiss

Oxides	Sp. No. 64	Sp. No. 291	Sp. No. 79
TiO <sub>2</sub>	2.25	2.14	2.60
Fe <sub>2</sub> O <sub>3</sub>	7.30	7.32	7.52
FeO	17.42	18.96	22.03
MgO	5.99	4.26	3.16
Fe <sup>2+</sup> /Mg	1.60	2.91	3.80
N <sub>z</sub>	1.628	1.639	1.662

Analyst: B. P. Gupta.

The biotite is strongly pleochroic and the pleochroic scheme varies with the change of refractive index, viz., grains of lower refractive index have X = Yellow, Y = Yellowish brown, Z = Brown, while for grains with higher refractive index values the scheme is X = Brownish Yellow, Y = Brown, Z = Deep brown. However, the absorption is  $Z > Y > X$  in both cases. Pleochroic haloes around allanite inclusions within biotite are common. The proportion of biotite in the granite gneiss varies between 2.20 to 15.91%.

## DISCUSSION

Several attempts have been made to demonstrate the quantitative relationship between refractive indices and chemical composition of biotite. Hall (1941) calculated that refractive index of biotite increases by 0.0046 with 1% increase in TiO<sub>2</sub>. Heinrich (1946) obtained a well-defined curve by plotting the combined weight percentages of FeO + 2 (Fe<sub>2</sub>O<sub>3</sub> + TiO<sub>2</sub>) against the refractive indices (Fig. 1). It appeared that the effect of

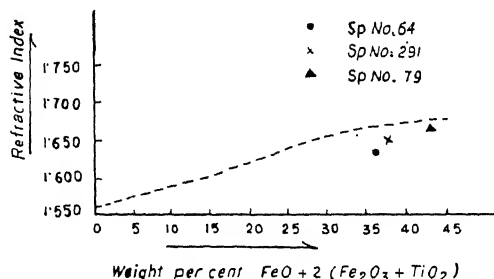


FIG. 1. Variation of refractive index of biotite with composition after Heinrich (1946).



$\text{Fe}^{3+}$  and Ti in increasing the refractive index is greater than that of  $\text{Fe}^{2+}$ . Both Hall and Heinrich concluded that no accurate correlation can be found between optical parameters and composition of phlogopite biotite series. For the present area samples, the plots of  $N_z$  and the parameter  $[\text{FeO} + 2(\text{Fe}_2\text{O}_3 + \text{TiO}_2)]$  (cf. Table II) fall reasonably close to Heinrich's (1946) curve (Fig. 1). But the three samples so plotted have similar proportions of  $\text{Fe}_2\text{O}_3$  and  $\text{TiO}_2$ , while the  $\text{Fe}^{2+}/\text{Mg}$  ratios are appreciably different. Thus, it might be suggested that in the present samples, the refractive index variation of biotite reflects at least to a large extent  $\text{Fe}^{2+}/\text{Mg}$  variation in biotite. As biotite is practically the only mafic mineral in the granite gneisses (except for traces of iron ore), it appears that the biotite refractive index variations in the present case reflects the  $\text{Fe}^{2+}/\text{Mg}$  ratio of the granite gneiss.

Polynomial trend surfaces upto fourth degree (cf. Krumbein, 1959) have been computed for the biotite refractive indices. The degree 4 map (Fig. 2) has taken into account 33.74% of the total variance. The corresponding deviation map (Fig. 3) shows a spotty pattern. There are several features worthy of note about the trend surface map (Fig. 2): (i) there is an overall lack of steep gradient, (ii) the trend lines do not parallel the regional foliation ( $S_1 = S_2$ ) but abut against the contact of the country rock and granite gneiss; (iii) there are two zones of high values in the east and west, separated by a central zone of low values. It may also be noted that the zones of high biotite  $N_z$  values coincide with areas of late stage K-feldspathization (dotted areas in Fig. 2) which led to the development of K-feldspar megacrysts. It is significant that because of the addition of K-feldspar consequent on K-feldspathization, the proportion of biotite in such zones is lower (2.2 to 5.3%) than in the rest of the area. Also, since biotite is practically the only ferromagnesian mineral in this granite gneiss, whose biotite  $N_z$  is correlatable to its  $\text{Fe}^{2+}/\text{Mg}$  ratio, it appears that the granite gneiss in the areas of high K-feldspathization have relatively high  $\text{Fe}/\text{Mg}$  ratio.

Thus an iron metasomatism appears to have accompanied the potassium metasomatism in the area.

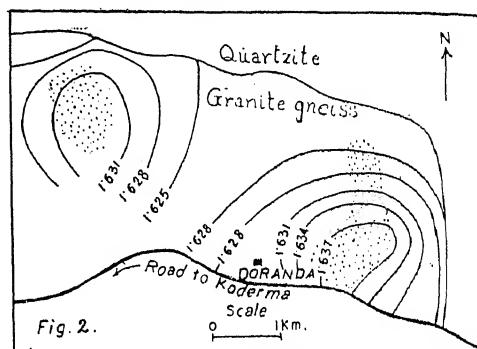


Fig. 2.

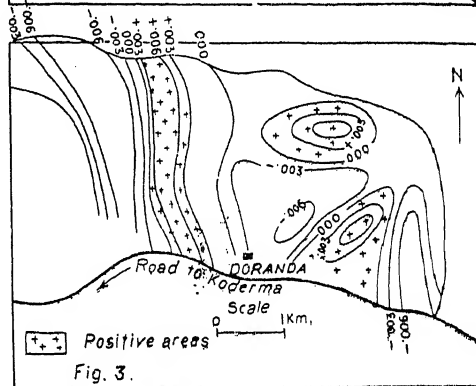


Fig. 3.

FIG. 2. Trend surface map of fourth degree. The dotted areas indicate zones of K-feldspathization.

FIG. 3. Deviation map of fourth degree surface.

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## LETTERS TO THE EDITOR

### STATIC PLANE SYMMETRIC SOLUTION OF A SCALAR-TENSOR THEORY IN A LYRA MANIFOLD

RECENTLY Sen and Dunn<sup>1</sup> have proposed a scalar-tensor theory of gravitation in a modified Riemannian manifold in which both the scalar and tensor fields have intrinsic geometrical significance. The scalar field in this theory is characterized by the function  $x^0 = x^0(x^i)$  where  $x^i$  are the coordinates in the four-dimensional Lyra manifold and the tensor field is identified with the metric tensor  $g_{ij}$  of the manifold.

The field equations given by Sen and Dunn<sup>1</sup> for the combined scalar and tensor fields are

$$R_{ij} - \frac{1}{2} g_{ij} R = -\delta\pi G (x^0)^{-2} T_{ij} + \omega (x^0)^{-2} \times (x^0, i x^0, j - \frac{1}{2} g_{ij} x^{0, k} x^{0, k}), \quad (1)$$

where  $\omega = 3/2$ ,  $T_{ij}$  is the energy-momentum tensor of the field,  $R_{ij}$  is the Ricci tensor and  $R$  is the usual Riemann curvature scalar. Sen and Dunn<sup>1</sup> and Halford<sup>2</sup> have obtained static spherically symmetric solutions of this theory. Furthermore Reddy<sup>3</sup> has shown that an analogue of Birkhoff's theorem of general relativity exists in this theory also. Recently the present author<sup>4</sup> has obtained exact cylindrical wave solutions of this scalar-tensor theory. This theory of gravitation has not been studied so far in the plane symmetric space-time of Taub<sup>5</sup>. We have, therefore, taken up the investigation of the vacuum field equations of this scalar-tensor theory in the plane symmetric space-time of Taub and have obtained an exact static solution which reduces to the Taub's empty space-time static solution of Einstein's theory<sup>5</sup>.

The field equations in the matter-free region are

$$R_{ij} - \frac{1}{2} g_{ij} R = \omega (x^0)^{-2} (x^0, i x^0, j - \frac{1}{2} g_{ij} x^{0, k} x^{0, k}), \quad (2)$$

where  $\omega = 3/2$ . We consider Taub's plane symmetric metric given by

$$ds^2 = -e^a [(dx^1)^2 - (dx^2)^2] - e^\gamma [(dx^3)^2 + (dx^4)^2], \quad (3)$$

where  $a$  and  $\gamma$  are functions of  $x^1$  and  $x^4$  only and  $x^1, x^2, x^3$  denote space coordinates whereas  $x^4$  corresponds to the time coordinate  $t$ . The plane symmetry assumed obviously implies that the scalar field  $x^0$  also is a function of  $x^1$  and  $x^4$  only.

In this note we restrict ourselves to the static case. Thus all the functions involved are independent of  $x^4$  ( $\equiv t$ ) and depend on only one space

coordinate  $x^1$ . With metric (3) in the static case the field equations (2) reduce to the following ordinary differential equations:

$$\gamma_{11} + \gamma_1^2 = 0, \quad (4)$$

$$a_{11} - \frac{1}{2} \gamma_1^2 = h_1^2, \quad (5)$$

$$\frac{1}{2} \gamma_1^2 + a_1 \gamma_1 = -h_1^2, \quad (6)$$

where we have put

$$x^0 = e^h | \sqrt{\omega}. \quad (7)$$

The lower suffix 1 after an unknown function denotes partial differentiation with respect to  $x^1$ .

From equations (4)–(6) one can easily obtain the solutions given by

$$\gamma = \log(k_1 x^1 + k_2), \quad (8)$$

$$a = (k_3/k_1) \log(k_1 x^1 + k_2) + k_4, \quad (9)$$

$$h = [-(2k_3 + k_1)/2k_1] \log(k_1 x^1 + k_2) + k_5; \quad (10)$$

where  $k_1, k_2, k_3, k_4$  and  $k_5$  are arbitrary constants. In order that  $h$  in (10) be real it is necessary that  $k_3 < k_1$ . Hence, for  $k_3 < k_1$ , the equations (8), (9) and (10) represent a static plane symmetric solution of vacuum field equations of Sen and Dunn's scalar-tensor theory.

If we choose  $k_4 = 0$  and  $k_3 = -k_1$  the above solution reduces to

$$\gamma = \log(k_1 x^1 + k_2), \quad a = -\frac{1}{2} \log(k_1 x^1 + k_2), \quad h = \text{constant, i.e., } x^0 = \text{constant}. \quad (11)$$

This is Taub's static plane symmetric solution of Einstein's empty space field equations.

It may be noted that like Taub's solution of Einstein's field equations our solution of Sen and Dunn's theory also has a singularity at  $x^1 = -k_2/k_1$ .

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## ON THE GRÜNEISEN PARAMETERS OF SOME FERRITES

THE anharmonic properties of solids are described in terms of the Grüneisen parameter,  $\gamma$  which relates the thermal expansion to the specific heat through

$$\gamma = 3\alpha V/k_T C_v = 3\alpha V/k_s C_p$$

where  $\alpha$  is the coefficient of linear expansion,  $k_T$  and  $k_s$  are isothermal and adiabatic compressibilities and  $C_v$  and  $C_p$ , the specific heats at constant volume and constant pressure respectively. This relation can be obtained from the well-known equation of state which is largely due to Mie<sup>1</sup> and Grüneisen<sup>2</sup>. The present note reports  $\gamma$  obtained from the measured values of lattice properties ( $\alpha$ ,  $C_p$  and  $k_s$ ) on the same ferrite samples. Further, variation of  $\gamma$  with temperature is also discussed in the case of these complicated ferrites.

The composite piezo-electric oscillator technique<sup>3</sup> has been used to determine the elastic constants of nickel-zinc, manganese-zinc and barium ferrites and the results have been reported elsewhere<sup>4</sup>. The thermal expansion<sup>5</sup> of these ferrites has been determined with two-terminal capacitance dilatometer<sup>6</sup>. Heat capacities<sup>7</sup> have been measured from 80° K to 303° K with a low temperature adiabatic calorimeter.

The Grüneisen parameters have been evaluated from the above lattice properties and the same are presented in Table I.

TABLE I

Grüneisen parameters of ferrites at 80° K and 303° K

Ferrite sample	80° K	303° K
NiZn (N)	0.51	0.53
NiZn (M)	0.36	0.39
MnZn	1.09	0.58
Ba	1.01	1.07

NiZn (N) : NPL (New Delhi) Sample.

NiZn (M) : Mullard (U.K.) Sample.

In the case of Ni-Zn ferrites, as can be seen from Table I, the Grüneisen gamma has different values at the same temperature. This change in  $\gamma$  is due to different molecular compositions of the two ferrite samples.

No comparison could be made between these values and the predicted ones based on the calculations from the lattice dynamical models since such data are not available in the literature. In the absence of such data, it is believed, the Grüneisen gammas obtained here from the experimentally determined quantities of thermal expansion, heat capacity and elastic constants are highly reliable

and useful as the same samples have been used in collecting the relevant data from experiments.

The nature of temperature variation of  $\gamma$  has been studied by many workers for simple solids and in general  $\gamma$  increases with increasing temperatures. The present study of variation of  $\gamma$  with temperature confirms the same. The change in  $\gamma$  from 80° K to 303° K is small (about 8%) for all ferrites except in the case of MnZn ferrite. However, rapid changes in  $\gamma$  might take place at still lower temperatures, i.e., at temperatures around  $0.2\theta_D$  ( $\theta_D$  being the Debye temperature) as predicted by Barron<sup>8</sup>. This could not be checked in the present case as cryogenic facilities are not available to go below liquid air temperature.

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## OXIDATION OF PHOSPHORUS PENTA- SULPHIDE WITH POTASSIUM PERMANGANATE AND POTASSIUM FERRICYANIDE: AN AMPEROMETRIC STUDY

THE increasing use of phosphorus pentasulphide in various industries such as production of oil additives, ore flotation agents, insecticides<sup>1</sup>, etc., has aroused considerable interest in the standardization of aqueous phosphorus pentasulphide. In the present communication an amperometric method has been adopted for the oxidimetric determination of phosphorus pentasulphide using potassium permanganate and potassium ferricyanide in alkaline medium.

Stock solution of phosphorus pentasulphide was prepared from E. Merck's guaranteed reagent in 1N aqueous solution of sodium hydroxide and standardized by subjecting aliquots to elemental analysis for sulphur<sup>2</sup>. A pure recrystallised sample of potassium ferricyanide was used. Potassium permanganate used was of BDH AnalaR grade and standardized by oxalate method. Sodium arsenite was prepared from E. Merck's arsenious oxide by

dissolving in minimum quantity of sodium hydroxide and neutralized with dilute hydrochloric acid. The strength was further checked against bromate. All other reagents used were of BDH AnalaR grade.

A manual amperometric unit as described by Kolthoff *et al.*<sup>3</sup> was set up. Titrations were carried out using rotating platinum electrode as polarizable electrode and saturated calomel electrode as reference electrode.

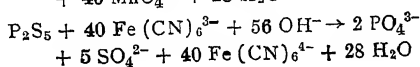
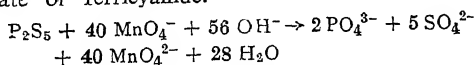
In a series of experiments an aliquot of phosphorus pentasulphide was taken in a beaker and a known excess of potassium permanganate or potassium ferricyanide was added. Sodium hydroxide was added to maintain the overall alkali concentration between 2 M–3 M. In the case of titration with ferricyanide osmium tetroxide (2 drops) was also added. The mixtures were kept aside at room temperature for about 30 minutes to ensure complete oxidation. The remaining excess of permanganate or ferricyanide was then back-titrated with standard arsenite solution amperometrically at an applied potential of +0.1 V vs. S.C.E. The end-point was located graphically as the point of intersection of two straight lines and L-shaped curve was obtained. A blank titration was also run simultaneously. From these observations the amount of permanganate or ferricyanide consumed during the oxidation and molar equivalent of oxidant per mole of phosphorus pentasulphide were calculated. These results are presented in Table I.

TABLE I

Phosphorus pentasulphide conc. 0.005 M  
Potassium permanganate conc. 0.15 M  
Potassium ferricyanide conc. 0.12 M  
Overall alkali conc. 2–3 M

P <sub>2</sub> S <sub>5</sub> taken ml	Potassium permanganate consumed, ml	Potassium ferricyanide consumed, ml	Number of equivalents of oxidant consumed per mole of P <sub>2</sub> S <sub>5</sub>	
			Potassium permanganate	Potassium ferricyanide
1.0	1.34	1.66	40.20	39.84
2.0	2.66	3.34	39.90	40.08
3.0	4.00	5.00	40.00	40.00
4.0	5.34	6.66	40.05	39.96
5.0	6.68	8.34	40.08	40.03

The results (Table I) reveal that 40 equivalents of permanganate or ferricyanide are required for 1 mole of phosphorus pentasulphide. The following mechanism is therefore suggested for the oxidation of phosphorus pentasulphide by permanganate or ferricyanide.



Thus, the equivalent weight of phosphorus pentasulphide under these conditions has been found to be 1/40 of the molecular weight. It may be noted that the procedure is simple, accurate and rapid.

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### CHEMICAL COMPONENTS OF *PTEROCARPUS SANTALINUS* SAPWOOD

THE sapwood of *Pterocarpus santalinus* constitutes one-fourth of the whole wood and does not seem to have been put to any proper use. In this connection a knowledge of its components will be useful. It is easily separated for study. Reports on the components of the heartwood and bark have already been made<sup>1,2</sup>.

The sapwood shavings (3 kg) were extracted by boiling with: (i) light petroleum (3 × 5 hr), (ii) chloroform (3 × 5 hr) and (iii) ethanol (3 × 6 hr) in succession. Light petroleum extract contained terpenoids mainly. The chloroform and the alcohol extracts on concentration gave deep red viscous liquids which did not show any marked difference in their composition on T.L.C examination and were therefore mixed for study.

(i) *Light petroleum extract*.—The concentrate was subjected to column chromatography over silica gel eluted first with light petroleum and continued with light petroleum: C<sub>6</sub>H<sub>6</sub> (3:1), light petroleum: C<sub>6</sub>H<sub>6</sub> (1:1) and pure C<sub>6</sub>H<sub>6</sub>. Three compounds A, B and C were obtained and were characterised as follows.

*Compound A*.—It crystallised from MeOH as colourless tubes (200 mg), m.p. 226–8°, [α]<sub>D</sub><sup>20</sup> –61.5° (C, 0.9, CHCl<sub>3</sub>). It gave +ve LB and TNM tests and was identified as acetyl oleanolic aldehyde by its IR and NMR and confirmed by comparison with an authentic sample (m.p. T.L.C. and I.R.). It would appear that this compound does not occur outside the family, Leguminosae

and is of chemotaxonomic value. This sapwood is a convenient source for its isolation.

**Compound B.**—It formed colourless needles from MeOH (300 mg), m.p. 266–8°,  $[\alpha]_D^{20} + 72.0^\circ$  (C, 0.8,  $\text{CHCl}_3$ ). It gave +ve LB and TNM tests and was identified as acetyl oleanolic acid by direct comparison of its methyl ester with authentic sample of acetyl oleanolic acid methyl ester (m.p., T.L.C., I.R.).

**Compound C.**—It crystallised from MeOH as colourless needles (100 mg) m.p. 278–9°,  $[\alpha]_D^{20} + 76.5^\circ$  (C, 1.1,  $\text{CHCl}_3$ ). It answered LB and TNM tests and was identified as erythrodiol by its IR and NMR and the identity confirmed by direct comparison with an authentic sample (m.p., T.L.C., I.R.).

**$\text{CHCl}_3$  and EtOH extracts.**—The deep red residue obtained on combining the two extracts and subsequent removal of the solvents was dissolved in a small amount of hot  $\text{C}_6\text{H}_6$  and left overnight in the refrigerator when it deposited a solid which was filtered and repeatedly crystallised from  $\text{C}_6\text{H}_6$  till it gave constant m.p. The m.p. 104° was undepressed when admixed with pterocarpol obtained from the heartwood; identity was supported by T.L.C. and I.R.

The first mother liquor showed three major red pigments on T.L.C.; it was chromatographed over polyamide column. Elution with  $\text{CHCl}_3$  : MeOH (25 : 1) and  $\text{CHCl}_3$  : MeOH (25 : 2) gave two red pigments D and E.

**Compound D.**—It formed red needles (120 mg) from aq. MeOH, m.p. 294°.  $\lambda_{\text{max}}^{\text{MeOH}}$  : 238, 280, 320, 472 and 504 nm. This was identical with a sample of santalin-B obtained from the heartwood (T.L.C. and mixed m.p.).

**Compound E.**—It came out of aq. MeOH as red needles (140 mg), m.p. 302–303° and showed UV absorptions identical with those of santalin-A. The identity was confirmed by direct comparison with a sample obtained from the heartwood (T.L.C., m.m.p.).

The co-occurrence of the related compounds erythrodiol ( $-\text{CH}_2\text{OH}$ ), acetyl oleanolic aldehyde ( $-\text{CHO}$ ) and acetyl oleanolic acid ( $-\text{COOH}$ ) is somewhat special in the sapwood and would be of biosynthetic interest. The presence of the corresponding compounds of the lupane<sup>3</sup> and ursane series<sup>4</sup> has been reported in other sources.

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#### EFFECT OF CERTAIN PHENOLIC COMPOUNDS ON THE GROWTH OF THREE ISOLATES OF *P. ORYZAE*

BLAST disease of rice (*Oryza sativa* L.) caused by *Piricularia oryzae* Cav. is destructive in most areas of the world<sup>1</sup>. Breeding programme to develop blast resistant varieties of rice for the control of the disease has become complicated with the occurrence of races of the pathogen<sup>2</sup>. Phenolic compounds have long been implicated in the resistance of plants against parasites<sup>3</sup>. Compounds such as *p*-hydroxy benzoic, protocatechuic and vanillic acids ( $\text{C}_6-\text{C}_1$  phenolics) and *p*-coumaric, caffeic and ferulic acids ( $\text{C}_6-\text{C}_3$  phenolics) were reported to occur consistently in rice plants<sup>4,5</sup>. The results on the effect of these compounds on the growth of three isolates of *P. oryzae*, viz., races AP37, M5 and M4 which belong to the International race groups IE. 4, IF. 3 and IC. 6 respectively (Chakrabarthy, C.R.R.I., Cuttack—personal communication) are reported here.

Czapek's broth, modified by adding 3 g of sodium nitrate, 1 mg of thiamine and 5  $\mu\text{g}$  of biotin per litre, was distributed in 50 ml quantities into 250 ml Erlenmeyer flasks. Each of the phenolic compound, dissolved in small quantity of absolute ethanol, was added separately to the flasks, in duplicate, to obtain concentrations ranging from  $10^{-4}$  to  $10^{-2}$  M, pH was adjusted to 7.0, sterilized at 110° C for 15 min and inoculated with 8 mm discs of the actively growing (7 days old) cultures of the three isolates from Czapek's agar plates. The flasks were incubated for 14 days at room temperature ( $28 \pm 1^\circ \text{C}$ ), the mycelial dry weights determined and the average values were calculated.

The results (Table I) showed that growth of three isolates of *P. oryzae* was inhibited by the phenols tested. The  $\text{C}_6-\text{C}_3$  phenolic compounds (with  $\text{CH}=\text{CH}-\text{COOH}$  group attached to benzene ring), excepting *p*-coumaric acid, were found to be more toxic than  $\text{C}_6-\text{C}_1$  phenolics. Srinivasan and Narasimhan<sup>6</sup> noted that dihydroxy phenolic acids were more inhibitory to *Colletotrichum falcatum* than the corresponding monohydroxy phenolic acids. In the present study also, caffeic acid was found to be more inhibitory to *P. oryzae* isolates than the corresponding monohydroxy phenol, viz., *p*-coumaric acid. However, protocatechuic acid

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TABLE I  
Effect of six phenolic acids on the growth\* of *P. oryzae* races AP 37, M5 and M4

Phenol	Race	Concentration (M)					
		$10^{-4}$	$10^{-3}$	$2 \times 10^{-3}$	$4 \times 10^{-3}$	$6 \times 10^{-3}$	$10^{-2}$
<i>p</i> -Hydroxybenzoic acid	AP37	256.5	182.1	117.9	56.8	0	0
do.	M5	241.2	159.0	100.0	36.6	0	0
do.	M4	203.1	156.0	88.5	35.0	0	0
Protocatechuic acid	AP37	218.8	185.8	129.8	80.3	50.6	0
do.	M5	242.1	170.9	90.7	52.2	39.7	0
do.	M4	198.2	162.5	141.5	39.1	35.4	0
Vanillic acid	AP37	229.6	189.8	130.3	93.1	53.4	0
do.	M5	203.2	191.3	97.0	87.2	46.7	0
do.	M4	205.2	180.3	89.1	87.6	44.4	0
<i>p</i> -Coumaric acid	AP37	244.4	222.7	181.9	128.5	100.6	39.6
do.	M5	210.0	207.0	165.6	112.9	95.5	25.0
do.	M4	199.1	166.8	102.4	54.8	30.0	19.4
Caffeic acid	AP37	176.8	132.0	32.7	8.6	0	0
do.	M5	141.7	81.5	23.4	0	0	0
do.	M4	65.3	26.9	14.6	0	0	0
Ferulic acid	AP37	224.0	205.2	123.7	46.1	18.5	0
do.	M5	189.2	127.0	79.3	28.4	0	0
do.	M4	148.0	120.0	56.6	23.8	0	0
Control	.. AP37	224.4;	M5—212.0;	M4—202.6			

\* Mycelial dry weight in mg/50 ml of culture broth.

and its corresponding monohydroxy phenol, *p*-hydroxybenzoic acid were relatively similar in their toxic effect towards the *P. oryzae* isolates. Vanillic and ferulic acids proved to be less toxic than the dihydroxy phenolics, viz., protocatechuic and caffeic acids, to all the isolates and thus revealed that methylation of a -OH group of dihydroxy phenols decreased their toxicity. Srinivasan and Narasimhan<sup>6</sup> and Greathouse and Rigler<sup>7</sup> obtained similar results with other fungi. However, ferulic acid was more inhibitory than *p*-coumaric acid to the three isolates of *P. oryzae*.

Based on their reactions on rice varieties, the races AP37, M5 and M4 were classified as virulent, moderately virulent and less virulent respectively<sup>8</sup>. At the lowest concentration of phenols tested ( $10^{-4}$  M), *p*-hydroxy benzoic and *p*-coumaric acids and *p*-hydroxy benzoic and protocatechuic acids have stimulatory effect on the growth of races AP37 and M5 as was noted by Kuwatsuka<sup>4</sup> in case of *p*-hydroxy benzoic, vanillic, *p*-coumaric, ferulic and chlorogenic acids at  $10^{-3}$  M with an isolate of *P. oryzae*. These compounds might have possibly been metabolized by the respective races of *P. oryzae* as in the case of other fungi<sup>9,10</sup>. In fact Wakimoto *et al.*<sup>11</sup> claimed that catechol, caffeic acid, gallic acid and quercetin at  $10^{-3}$  M inhibited the growth but both rutin ( $10^{-3}$  M) and quercetin ( $10^{-4}$  M) were decomposed by the fungus into protocatechuic acid and phloroglucinol. In general, the toxicity of all phenols towards the three races of *P. oryzae* tested, increased with increase in concentration. At

concentrations between  $10^{-4}$  to  $4 \times 10^{-3}$  M the virulent race exhibited relatively more tolerance than other races. The less virulent race was found to be highly sensitive towards the toxic activity of phenols. On the other hand, the sensitivity of moderately virulent strain was intermediary. Hence, it can be concluded that the races of *P. oryzae* differed in their tolerance to the toxic effect of phenols at the above concentrations. However, at higher concentrations ( $6 \times 10^{-3}$  M and  $10^{-2}$  M) all the races were inhibited almost to a similar extent.

Caffeic and ferulic acids proved to be effective inhibitors of the three races of *P. oryzae* *in vitro*. The same compounds were reported to play a major role in the resistant reaction of rice plants towards the blast fungus<sup>4,5</sup>. Hence it is suggested that the degree or the concentration to which these phenols were synthesized in rice varieties in response to *P. oryzae* infection will determine the extent to which a particular race can parasitize the host tissues.

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#### NEW EXPOSURES OF TALCHIR STRIATED PAVEMENTS IN MADHYA PRADESH

THE authors while studying the Permian deposits in Son Valley (Madhya Pradesh) came across two striated pavements not hitherto reported. One of the pavements (Fig. 1—I) is exposed from underneath the marine fossil beds 1, 2 near Manendragarh ( $82^{\circ} 12' : 23^{\circ} 13'$ ) and the other pavement (Fig. 1—II) is exposed on the right bank of Gejnala near Baikunthpur ( $82^{\circ} 35' : 23^{\circ} 15'$ ), about 50 km east of the first exposure.

North-east of the Hasdo railway bridge, underneath the Manendragarh marine fossil bed, the granite surface has glacial striations, flutings and crescentic gouge marks. The striae (Fig. 2) are

best developed on the north-eastern face of the granite exposure, and are oriented south-east to north-west ( $315^{\circ}$ ) with pinheads in the south-east. Fluting, though often not very deep, follows the same general direction. Along a south-east vertical face of the granite which can only be seen from the river bed, several crescentic gouge marks were seen. Five of these marks are in a line and only few centimetres apart (Fig. 3), whereas the others are scattered on the same face. The convex deep cut of the gouges were obviously made by a glacier, moving from south-east. On the south-east side of the ridge, granite blocks were plucked and the edges rounded by the ascending ice. This granite block may have been a crag and tail structure in the path of the ice. It is, therefore, concluded that at this spot the direction of the glacier movement was from south-east to north-west.

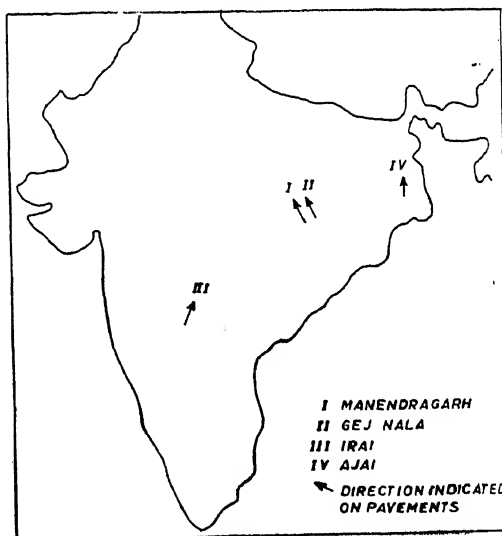
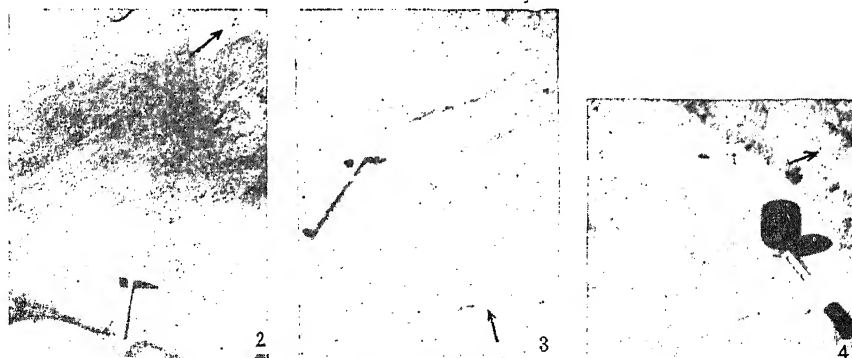


FIG. 1. Ice movement direction in India as shown by known striated pavements.



FIGS. 2-4. Fig. 2. Striated pavement—Manendragarh. Fig. 3. Crescentic gouge marks—Manendragarh. Fig. 4. Striated pavement—Gejnala.

The other pavement was seen along right bank of the Gejnala near Baikunthpur. This exposure is about 200 metres upstream from Gejnala and the Jhunka nala junction and it can be reached easily by following the Jhunka nala downstream from the road bridge.

About five metres above the Gejnala bed, a tillite bed is resting on the striated granite pavement (Fig. 4). The striations are very fine with pinheads towards south-east. No other markings were seen on this pavement. The markings indicate a south-east to north-west ( $322^\circ$ ) direction of the ice movement.

This granite exposure is elongated in the direction of the ice movement, it is sloping upstream and may have been a *rochemoulonnee*.

The present study, therefore, indicates that in the Son valley the glaciers were moving generally from south-east and not from north-west as deduced by Ghosh and Mitra<sup>3</sup>.

Only two other striated pavements have so far been reported from India, one is at Irai<sup>4</sup> (Fig. 1—III) in Pranhita-Godavari Valley (re-examined by Smith<sup>5</sup>), showing south-west-north-east direction of the ice movement and the second is on the Ajai river bank<sup>6</sup> (Fig. 1—IV) showing south to north direction of ice movement. These two new exposures from Madhya Pradesh conform with the general direction of ice movement indicated by the two earlier known pavements in India during Talchir Period.

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## GOITROGENIC ACTIVITY OF DANAZOL— A SYNTHETIC STEROID DERIVATIVE

17  $\alpha$ -PREGN-4-EN-20-YNO-(2, 3-OL) isoxazol-17ol (Danazol), clinically the compound was found to be of interest in the management of endometriosis, precocious puberty and various breast disorders<sup>1</sup>.

To investigate the effect of this compound on pituitary thyrotrophic function, the present studies were taken. The effect of the goitrogenic action of danazol was investigated in female desert rat (gerbil) and mice. The criteria used in this investigation were:

1. Thyroid follicular epithelial cell height and its microscopic structure.
2. Collection of radioactive iodine by the thyroid gland.
3. Protein-bound radioiodine ( $\text{PbI}^{131}$ ) conversion rate.

A group of 10 female gerbils (*Meriones hurrianae* Jerdon) weighing  $69 \pm 5$  g were injected with danazol subcutaneously, suspended in olive oil in daily doses of 3 mg/day for a period of 66 days. Control animals received daily injection of vehicle alone. The animals were given rat food and water *ad libitum*.

*Mouse (Swiss albino strain).*—A group of ten mice weighing  $30 \pm 1$  g were injected with danazol 1 mg/day for a period of 70 days. The drug was injected in the pelvic region. The mice were given rat food, wet gram and water *ad libitum* in the laboratory at  $23 \pm 1^\circ\text{C}$  with 10 hr illumination. An equal number of controls received olive oil.

An injection of carrier free Na  $\text{I}^{131}$  was given i.p. (in a dose of  $5 \mu\text{Ci}$  per mice and  $10 \mu\text{Ci}$  per gerbil, contained in a volume of 0.5 ml). After 48 hrs the animals were sacrificed and 2–3 ml of blood were withdrawn into a heparinized syringe from the vena cava under ether anaesthesia. Following withdrawal of blood, the thyroid with underlying trachea was removed, dissected free of fat, and connective tissue and weighed on a Mettler's balance. Counting of  $\text{I}^{131}$  was done in a G-M counter (Nuclear Schicago Model 180 B).

Protein bound radioiodine ( $\text{PbI}^{131}$ ) was determined according to the method of Ghosh *et al.*<sup>2</sup>. The conversion ratio was calculated as follows<sup>3</sup>:

$$\text{CR} = \frac{\text{serum Pb I}^{131} \text{ cpm}}{\text{serum Pb I}^{131} \text{ cpm} + \text{serum I}^{131} \text{ cpm}} \times 100.$$

It is seen in Table I that the total radioactivity in the thyroid gland is significantly higher in the danazol-treated mice and gerbil than in the controls. This denotes a decrease in the rate of discharge of thyroid hormone after danazol administration.

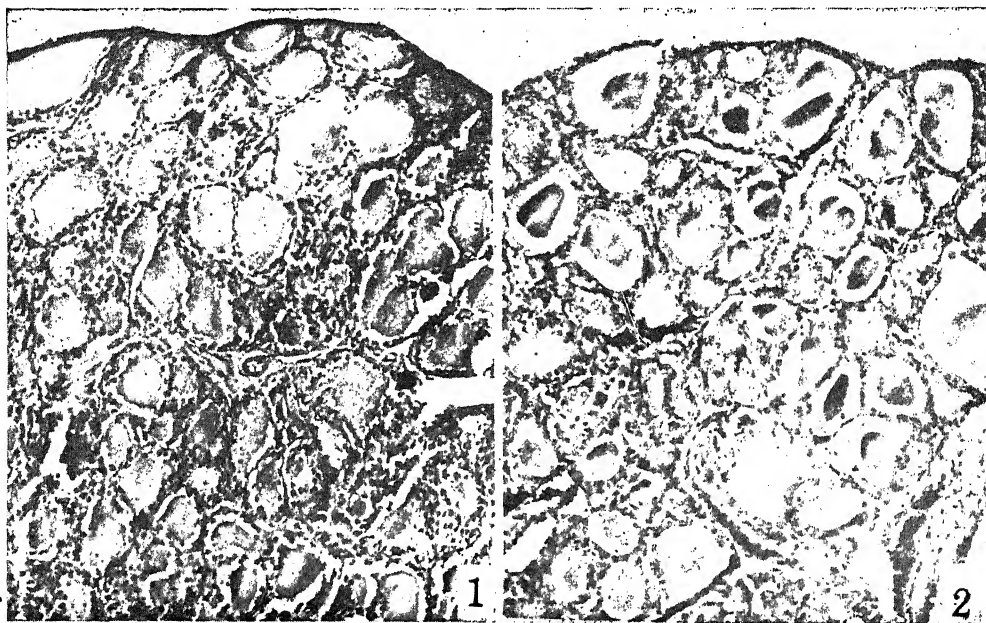


TABLE I  
Effect of danazol on the thyroid radioiodine uptake

Group No.	Treatment	No. of days	Total dose (mg)	Body weight (g)	Thyroid weight (mg/100 g body weight)	$I^{131}$ uptake (c/min/thyroid)	Normal (%)	CR (%)
1.	Gerbil Control	..	..	62±4	9.2±0.8	28273	100	58
2.	Danazol	70	200	69±5	11.3±0.5†	58952*	208*	44*
3.	Mice Control	..	..	24±2	9.1±1.2	3267	100	..
4.	Danazol	70	70	30±1	13.2±1.2‡	7467†	228†	..

Body weights and weights of thyroid : means of ten determinations.

\*  $P < 0.01$  compared with group 1, †  $P < 0.01$  compared with group 3, ‡ Non-significant compared with control.



FIGS. 1-2. Fig. 1. Thyroid follicles of a control gerbil, HE  $\times 80$ . Fig. 2. Thyroid follicles showing depletion of colloidal material (Danazol 200 mg HE,  $\times 80$ ).

The rate of conversion of administered inorganic radioiodine of the plasma has been used to evaluate thyroid activity following danazol administration. Danazol produced a significant decrease in the protein bound radioiodine conversion rate (Table I). This denotes that danazol acts on thyroid gland function directly.

**Thyroid microscopic structure.**—Danazol treatment in mice and gerbil induced marked atrophic changes in the microscopic thyroid structure (Figs. 1 & 2). Prolonged administration of danazol caused liquefaction and vacuolation of the thyroid follicles. The follicular epithelium presents high cuboidal cells. The secretory epithelium is hypertrophic and contains swollen nuclei. The average height of the follicular epithelium is increased

significantly ( $P < 0.01$ ) (Danazol : mean epithelial height of the thyroid follicle cells;  $11.64 \pm 0.2 \mu$ ; control :  $9.12 \pm 0.25 \mu$ ). Colloidal material of the thyroid follicle became dilute and highly vacuolated. The largest glands were virtually devoid of colloid.

Goitrogenic action of danazol is comparable with the action of antithyroid drugs, viz., methyl thiouracil/propyl thiouracil in rat<sup>4</sup>, guinea pig<sup>5</sup> and metopiron (SU-4885) in gerbil<sup>6</sup>. Increased thyroid follicular epithelial cell height has been used as an index for goitrogenic action. In the present investigation goitrogenic action of danazol has been confirmed using thyroid epithelial cell height and histological structure as an index.

The  $I^{131}$  content of the thyroid gland was significantly higher in the danazol treated groups than in the controls. This was interpreted as being the result of a decrease in the rate of discharge of thyroid hormone, which further supports the supposition of the inhibition of thyroid function after danazol administration. In addition a direct effect on the trapping and binding of iodine by the thyroid gland is reflected in a decreased  $Pb\ I^{131}$  conversion rate. The evidence is quite conclusive that danazol acts on thyroid gland function directly as well as by influencing pituitary thyrotrophic activity in enhancing  $I^{131}$  uptake.

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#### NITRATE REDUCTASE IN WILD AND CULTIVATED WHEATS

RECENT studies on photosynthetic rates in wild and cultivated wheats have shown that the primitive wheats have higher photosynthetic rate than the cultivated types<sup>1,2</sup>. This behaviour was found to be true during vegetative phase even at the photo-phosphorylation level<sup>3</sup>. Tsunoda<sup>4</sup> has further shown that the photosynthetic rates are strongly correlated with leaf nitrogen. Croy and Hageman<sup>5</sup> have shown a strong correlation between the activity of nitrate reductase and total reduced nitrogen in wheat. Would then, selection for nitrate reductase help in selecting for high photosynthesis also? We here report the activity of nitrate reductase in diploid, tetraploid and hexaploid wheats grown under identical conditions. Twenty-eight genotypes, belonging to ten different species and including all the basic genomes of wheat, were raised in sand culture. Hoagland's nutrient solution at full strength was supplied at weekly interval<sup>6</sup>. Fully expanded leaves of one-month old seedlings

were assayed for nitrate reductase using the *in vivo* method<sup>7,8</sup>. There were three replicates for each genotype and for each determination 200 mg leaf material obtained from several leaves was used. In a preliminary experiment the effect of glucose, sucrose and 3-phosphoglyceric acid was determined on the *in vivo* activity of nitrate reductase. Only 1% glucose was found effective in raising the NR activity. Therefore, NR activity in all genotypes was determined in the presence of glucose.

TABLE I  
Nitrate reductase activity *in vivo* in leaves in different genotypes of one-month old wheat seedlings

Species	Genome	Culture	$\mu\text{moles NO}_2^-/\text{g.f.w./h}$
		Code Name	
<i>T. monococcum</i>	AA	..	4.92
<i>T. speltoides</i>	BB	..	4.33
<i>T. tauschii</i>	DD	..	5.04
<i>T. carthlicum</i>	AABB	Parent II	2.92
		5	2.80
		39854	2.92
<i>T. dicoccum</i>	AABB	NP 202	1.92
<i>T. durum</i>	AABB	Parent VIII	
		HD 4502	2.75
		NP 404	3.17
<i>T. polonicum</i>	AABB	Parent III	2.33
<i>T. turanicum</i>	AABB	Parent X	2.17
<i>T. turgidum</i>	AABB	18	2.75
		23	2.83
		24	2.50
		28	2.67
		43	2.67
		44	2.00
		50	2.33
		67	2.17
		68	2.25
		46432	2.92
<i>T. aestivum</i>	AABBDD	Lusitanium	2.08
		Parent V	
		Hira	2.25
		Kalyansona	2.17
		C-306	2.33
		Moti	2.58
		Karchia	2.33
		LSD at 5%	0.41

The highest enzyme activity was observed in three diploids *Triticum monococcum*, *T. speltoides* and *T. tauschii*. Amongst the 4 × types the lowest activity of 1.92  $\mu\text{moles NO}_2^- \text{g}^{-1} \text{hr}^{-1}$  was observed in *T. dicoccum* cv. NP 202, whereas the maximum was in *T. durum* cv NP 404 being 3.17  $\mu\text{moles}$

$\text{NO}_2^- \text{ g}^{-1} \text{ hr}^{-1}$ . Within one species such as *T. turoides*, the variation was from 2.00 to 2.92  $\mu\text{moles NO}_2^- \text{ g}^{-1} \text{ hr}^{-1}$ . In *T. aestivum*, the enzyme activity just varied from 2.17 to 2.58  $\mu\text{moles NO}_2^- \text{ g}^{-1} \text{ hr}^{-1}$ .

Therefore, it appears that only the  $2 \times$  genotypes have higher NR activity than  $4 \times$  and  $6 \times$  genotypes. There does not exist any clear distinction between  $4 \times$  and  $6 \times$  types in this regard although they differ with respect to photosynthesis<sup>1</sup>. This study shows that close relationship between total nitrogen and photosynthesis may not have much to do with nitrate reductase activity. Accordingly, the NR activity cannot be made an indirect index of photosynthetic activity also.

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#### SEX-RATIO OF *ORYZAEPHILUS MERCATOR*, FAUVEL ON DIFFERENT FEEDS

THE biology of *Oryzaephilus mercator* has been investigated by Howe<sup>4</sup> on wheat, coconut and grass meal; by Fraenkel and Blewett<sup>3</sup> on fig and pea; by Breese<sup>2</sup> on wheat, unrefined and crystal sugar; by Thomas and Shepard<sup>6</sup> on walnut, oat and raisin, and by Back and Cotton<sup>1</sup> on split maize. The present author studied the life-history of *O. mercator* on different varieties of paddy, wheat and dry fruits. The results reveal that the life span of the weevil varies considerably on different feeds under similar conditions of temperature and humidity. The amount of consumption by *O. mercator* varies vastly on different feeds. As such it is expected that the sex-ratio of the insect might show variation on different feeds. The present investigation was undertaken to look into it experimentally.

To obtain the eggs of *O. mercator* a culture was raised on Haydak<sup>5</sup> formula. The meals under test included the dry fruits—groundnut, cashew, dates, raisin, coconut, almond, peanut and figs. The cereals selected for the experiments were gram, maize Ganga hybrid—I, wheat (Rs. 31-1) and rice (IR-8). The wheat and rice varieties selected were the most resistant varieties to the beetle. The cereals were powdered in a hammer mill and sieved through 60 meshes to an inch. The flour was conditioned at 30° C and 70% RH. for 10 days and resieved before use. The dry fruits were sliced to render them easily acceptable by the newly hatched larvae. A suitable quantity of each feed was placed in a glass vial of  $3 \times 1 \text{ cm}$  size. In each batch 90 eggs were taken. Three replications for each experiment were managed. Forty newly-emerged adults were randomly selected and sexed. On the feeds where the number of adults emerged was less than 40, all the adults were sexed.

On rice (IR-8 var.) the total number of males and females emerged was 50 and 70 respectively; whereas on wheat (Rs. 31-1) it was 52 and 68 respectively. On maize and gram meals, the number of males appeared was 57 and 68 respectively, and that of females 63 and 52 respectively. Table I gives the individuals accounted on different meals.

TABLE I  
Sex-ratio of *O. mercator* on various feeds

S. No.	Feeds	No. of insects per batch	Tests			Ratio
			I ♂/♀	II ♂/♀	III ♂/♀	
1	Rice (IR-8)	40	18/22	15/25	17/23	1.40
2	Wheat (Rs. 31-1)	40	20/20	17/23	15/25	1.30
3	Maize (G.Hy. 3)	40	19/21	18/22	20/20	1.10
4	Gram	40	21/19	23/17	24/16	0.76
5	Groundnut	40	16/24	17/23	15/25	1.50
6	Cashew	40	11/29	11/29	13/27	2.42
7	Dates	..	5/3	2/2	3/3	0.69
8	Raisin	..	4/2	2/1	3/3	0.66
9	Coconut	40	22/18	20/20	18/22	1.00
10	Almond	40	21/19	23/17	22/18	0.81
11	Peanut	40	15/25	18/22	16/24	1.44
12	Fig	..	15/11	9/8	12/11	0.83

The sex-ratio—female to male—on rice, maize, wheat and gram is 1.40, 1.10, 1.30 and 0.76 respectively. In other words, the number of females emerged on rice was the highest, and on gram the lowest amongst the cereals. On the contrary, the appearance of males was maximum on gram and minimum on rice.

On dry fruits, the number of females collected from groundnut, cashew, dates, raisin, coconut,

almond, peanut and fig was 72, 85, 9, 6, 60, 54, 71 and 30 respectively; and the males counted on the same meals were 48, 35, 13, 9, 60, 66, 49 and 36 respectively. On the above-mentioned meals the sex-ratio, i.e., female to male, is 1.50, 2.42, 0.69, 0.66, 1.00, 0.81, 1.44 and 0.83 respectively. The highest number of females was 85 on cashew and of males 66 on almond. The order of emergence of females to males, from the highest to the lowest on different dry fruits is as follows:

Cashew → groundnut → peanut → coconut → fig → almond → dates → raisin.

The foregoing results indicate that among the dry fruits, cashew showed the highest emergence of females and the lowest emergence of males; while almond exhibited maximum emergence of males and minimum emergence of females. On dates, raisin and fig 22, 15 and 66 adults appeared. The female to male ratio on these feeds is 0.69, 0.66 and 0.83 respectively, indicative of that the males on these feeds outnumber the females.

Comparing the sex-ratio of *O. mercator* on cereals with that on dry fruits, it is conclusive that the number of females developed on cashew is greater than that on rice. It is also significant that so far as the sex-ratio is concerned peanut and groundnut are much nearer to rice, wheat and maize. Similarly, gram is closer to dates and almond. The feeds employed in the present experiments, therefore, display a three fold categorisation based on the sex-ratio of the emerged weevils. The first category includes rice, wheat, maize, groundnut, cashew and peanut where the number of emerged females is higher than that of males. The second comprises gram, dates, raisin, almond and fig, where the number of males is greater than that of females. The third contains coconut only where the males and females are equal in number.

The experimental results indicate that on oil rich meals like cashew, coconut and almond, the sex-ratio varies as much as on carbohydrate-rich meals, viz., rice, maize, dates and raisin, which gives a clue to the fact that neither carbohydrate nor oil present in the meal affects the sex-ratio of the weevil. However, it would be interesting to find out the chemical factor responsible for the difference in the sex-ratio on different feeds.

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#### A NEW SPECIES OF *CRESEIS* (GASTROPODA, MOLLUSCA) FROM THE LACCADIVE SEA

THE genus *Creseis* (Fam. Cavoliniidae, Or. Thecosomata, Gastropoda) contains two common species. They are *C. acicula* Rang and *C. virgula* Rang. The former is considered as a monotypic species and the latter a polytypic species with four sub-species, namely, *C. v. virgula* (Rang), *C. v. clava* (Rang), *C. v. conica* (Eschscholtz) and *C. v. constricta* Chen and Bé. Among these four sub-species the first three were treated at the species rank by Rang (1828) and Eschscholtz (1829). Later on, Boas (1886) reduced them to sub-species on the basis of their morphological intergradations. They were considered as intermediate between the forms *C. acicula* and *C. virgula*, mainly on the basis of progressive curvature of the hind part of the shell. Chen and Bé (1964) erected a new sub-species *C. virgula constricta* on the basis of a constriction which separates the juvenile from the adult shell, considering the juvenile part of the shell as one of the major criteria for species identification. As the different size groups from the juvenile to adult stages of each sub-species were available in the International Indian Ocean Expedition collections, it was possible to revise the genus, assigning specific status to the sub-species (Sakthivel, 1972). In this context a new species obtained in the collections is described here.

Subclass	Opisthobranchia
Order	Thecosomata
Suborder	Euthecosomata
Family	Cavoliniidae d'Orbigny, 1888
Genus	<i>Creseis</i> Rang.

*Creseis bulgia* n. sp. (Figs. 1 and 2)

*Type locality*.—Off Kalpeni Island of the Laccadive Sea (10° 04' N, 73° 36' E).

*Dimensions*.—*Holotype*: Entire shell 2.20 mm long and 0.50 mm wide, Juvenile portion 0.45 mm long and 0.12 mm wide. *Holotype* is deposited in the Indian Ocean Biological Centre zooplankton museum (No. 10 BC-0239-06-36).

*Paratypes*:

1. 19° 04' N, 73° 36' E.  
Entire shell 2.0 mm long and 0.50 mm wide.

2. 20° 19' N, 38° 21' E.  
Entire shell 2.00 mm long and 0.50 mm wide.
3. 12° 41' S, 98° 25' E.  
Entire shell 2.1 mm long and 0.5 mm wide.
4. 29° 25' S, 109° 29' E.  
Entire shell 1.7 mm long and 0.45 mm wide.

Type specimens are deposited in the reference collections of Indian Ocean Biological Centre, Cochin.



FIGS. 1-2. Fig. 1. *Creseis bulgia*—Shell—Full view. Fig. 2. *Creseis bulgia*—Shell—Tip enlarged.

**Description.**—Shell is straight, conical and circular in cross-section. The total length of the adult shell is about four times more than the maximum diameter. The juvenile part of the shell has a distinct bulge at the apical end which separates the adult shell from the juvenile portion. Therefore the name *bulgia* is proposed to this new species.

**Remarks.**—*C. bulgia* has some resemblance to other species of *Creseis*, but differs distinctly in the juvenile portion of the adult shell. While the present species has a distinct bulge at the apical end of the juvenile portion with a length and width ratio of 4:1, *C. clava* has a spindle-shaped cylindrical juvenile portion with a length and width ratio of 5 to 6:1. The juvenile portion of the shell in *C. conica* is cylindrical with a reduced width at the tip. In addition the expansion growth (the increasing width from the juvenile to adult shell) and the length and width ratio (7:1) differ remarkably. *C. constricta* differs from *C. bulgia* in the constriction between the juvenile and adult portions of the shell, in the length and width ratio (6:1), and in the faint curvature of the hind end. *C. virgula* differs in the distinct curvature of the hind part of the shell.

**Distribution.**—The holotype was first located in the surface waters off the Kalpeni Island in the Laccadive Sea. The examination of the IIOE collections revealed that this species has wide

distribution from the Red Sea to the Australian Sea.

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#### SEASONAL VARIATIONS IN THE SIZE OF THE CAMBIAL INITIALS IN *POLYALTHIA LONGIFOLIA*

*Polyalthia longifolia* Hook. f. & Thoms., common avenue tree of the family Anonaceae, was selected to investigate the seasonal variations in the activity of cambium and changes in the size of the cambial initials. The present investigation like the earlier on *Dalbergia sissoo* (with storied cambial initials; Paliwal and Prasad<sup>2</sup>) is also aimed at determining whether or not the non-storied cambial initials of this tree undergo any seasonal variations in size. It was also intended to find out if any relationship exists between the variations in the size of the initials and the cambial activity.

The material was collected from a tree growing at the Delhi University Campus. Periodic collections were made from January to December, 1968, in the second week of every month. The material was fixed in Craff for 24 hrs. Blocks of 2.0 × 2.5 cm, containing portions of wood and bark, were obtained by using electric saw and single-edged blades. The blocks were sectioned in transverse and longitudinal planes with a Jung Wood Microtome, at 24–30 μ. Sections were stained using the lacmoid staining schedule as outlined by Cheadle *et al.*<sup>1</sup>. After passing the sections through the dehydration series, they were mounted in neutral Canada balsam.

As stated earlier the cambium is non-storied (non-stratified) and heterogeneous. The fusiform initials are long, tapering in outline, and are arranged irregularly. The rays are multiserial, with isodiametric initials. The fusiform initials show characteristically beaded cell walls. At the time of initiation of the cambial activity (May-June) there is only a slight decrease in the cell wall thickness of fusiform initials. The activity of cambium reaches its maximum in September as indicated by the number of cambial layers and their derivatives in transection.

The observations are based on the measurements for the fusiform and ray initials, periodically recorded and presented. The first visible change that occurs in the initials, at the time of cambial reactivation, is the decrease in cell wall thickening. Later, the fusiform initials elongate as well as enhance in breadth. Growth of the fusiform initials occurs in two phases. During the first phase, the length increases and in the second phase the breadth. At the time of cambium initiation there is increase in the length and breadth of the fusiform initials. The length of the fusiform initials reaches its maximum at the beginning of the active period whereas the breadth increases slowly and reaches the maximum at the end of the active period. The breadth of the ray initials increases sharply at the beginning of the active period, i.e., May-June.

A graphic representation of the fluctuations in light (duration of sunshine), temperature, rainfall, and relative humidity in Delhi during the year 1968 is presented in Fig. 1. It is evident that high temperature, high rainfall, high relative humidity, and short day conditions seem to increase the size of the cambial initials and also promote the cambial activity.

A comparison with *Dalbergia sissoo* reveals that in that tree decrease in the breadth and increase in the length of the fusiform initials takes place throughout the active period. As dormancy sets in these tend to become broader and a sharp fall in their length is recorded. In *Polyalthia longifolia*, on the other hand, the length of the fusiform initials enhances at the beginning of the active period and their breadth only when it tends to slow down. Furthermore, the breadth of the ray initials decreases sharply at the beginning of the active period, whereas it increases in the latter.

The data regarding the climatic changes in Delhi were obtained from the office of the Director-General of Observatories, Indian Meteorological Department, Lodi Road, New Delhi 110003. The

authors gratefully acknowledge their kind co-operation.

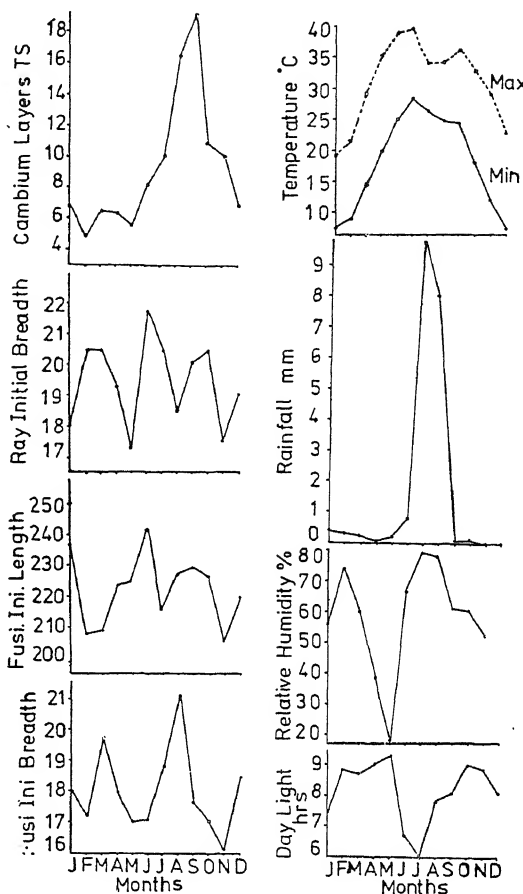


FIG. 1. Variations in the size (in  $\mu$ ) of the cambial initials in relation to various climatic factors.

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# EFFECTIVENESS OF DD-136, AN ENTOMO-PHILIC NEMATODE AGAINST INSECT PESTS OF AGRICULTURAL IMPORTANCE

THE potential use of nematodes in biological control of insects was discussed by Glaser and Wilcox<sup>2</sup>. It has now been established that DD-136 *Neoplectana carpocapse* Weiser offers scope for the natural control of insect populations in India (Yadava and Rao<sup>8</sup> and Mathur *et al.*<sup>4</sup>). Srivastava and Mathur<sup>7</sup>, Israel *et al.*<sup>3</sup> and Rao and Manju Nath<sup>6</sup> also tested the effectiveness of this parasitic nematode against some lepidopterous insect pests.

In the present work pathogenicity of the nematode on some agriculturally important insect pests has been tested under laboratory conditions (Table I). Nematode suspension was sprayed on

TABLE I

Population level of DD-136 from different insects after 9-10 days of trapping

Insects	Stage	Frequency (population size)	Corrected % mortality
<i>Athalia proxima</i> Klug	Larva	+	53.3
<i>Aulacophora foveicollis</i> Lucas	Adult	+++	66.6
<i>Dacus cucurbitae</i> Coquillett	Larva	—	..
<i>Diacrisia obliqua</i> Walker	"	+	53.3
<i>Dysdercus cingulatus</i> Fab.	Adult	++	58.2
<i>Epilachna vigintioctopunctata</i> (F.)	"	—	..
<i>Heliothis armigera</i> (Hb.)	Larva	++	58.2
<i>Leucinodes orbonalis</i> (Guen.)	"	++++	73.3
<i>Spodoptera litura</i> (Fabricius)	"	+++	66.6

Key to symbols: ++++ = Very high;  
+++ = High; ++ = Moderate;  
+ = Low; — = Absent.

five insects of about the same age with automizer at the rate of 125 nema per petridish. There were three replicates, the fourth being control sprayed with distilled water. The insects were allowed to feed on their natural host. The mortality of insects was counted after 24, 48 and 72 hr. The corrected mortality was calculated by Abbot's formula (Abbot<sup>1</sup>). Dead insects, if any, were removed after every count and kept for trapping of nematodes in 0.1% formalin solution. The population of nematodes was recorded in each host after 9-10 days of trapping. Dead insects from the control were also examined for the presence of nematode.

The highest mortality (73.3%) was recorded in *Leucinodes orbonalis* Guen., a new host for DD-136 nematode. It also produced the largest

numbers of nematodes. All test insects except *Dacus cucurbitae* Coq. and *Epilachna vigintioctopunctata* Fab. facilitated multiplication of nematodes (Table I).

The laboratory observations have confirmed that non-lepidopterous insects such as *Athalia proxima* Klug, *Aulacophora foveicollis*, Lucas and *Dysdercus cingulatus* Fab. are also susceptible to DD-136 nematode. Poinar<sup>5</sup> also listed some beetles as its hosts. Although it has been reported that DD-136 nematodes prefer lepidopterous insects but it is clear from the present investigation that this nematode can to some extent attack other orders of insects also which needs thorough checking for their pathogenicity. Mathur *et al.*<sup>4</sup> recorded *Diacrisia obliqua* Wik. as a host of DD-136 nematode. However, in the present investigations, only low population levels were recorded.

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## ADDITIONAL HOSTS OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

AMONG the phytonematodes the root-knot nematodes rank first for their notoriety with regard to the widest host range and worldwide distribution. These nematodes are predominant in areas where multiple cropping and intensive cultivation are practised. They produce galls, swellings, or knots on the roots of plants, giving a very unsightly look.



TABLE I

Sr. No.	Host	Family	Locality	Nature* and degree† of galling
1.	<i>Achyranthes aspera</i> L. var. <i>prophyristachya</i> Hook	Amranthaceae	Pipli (Sonepat)	Large; severe
2.	<i>Browallia</i> sp.	Solanaceae	Pipli (Sonepat)	Medium; severe
3.	<i>Cassia sophora</i> L.	Caesalpinaceae	Pipli (Sonepat)	Large; moderate
4.	<i>Cyperus rotundus</i> L.	Cyperaceae	HAU Farm, Hissar	Small; light
5.	<i>Dalbergia sissoo</i> Roxb.	Papilionaceae	Pipli (Sonepat)	Small; light
6.	<i>Euphorbia thymifolia</i> L.	Euphorbiaceae	Sonepat	Small; light
7.	<i>Nicotiana plumbaginifolia</i> Viv.	Solanaceae	Sisana (Rohtak)	Large; severe
8.	<i>Potamogeton</i> sp.	Potamogetonaceae	Pipli (Sonepat)	Small; moderate
9.	<i>Portulaca quadrifida</i>	Portulacaceae	HAU Farm, Hissar	Small; light
10.	<i>Withania somnifera</i> Dunal	Solanaceae	Sonepat	Medium; severe

\* Nature of gall: Small, medium, or large size. † Degree of galling: Light, moderate, or severe infestation.

They are responsible for tangible reduction in plant growth and yields.

In Haryana, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, is widely distributed. A survey of cultivated fields for root-knot nematode infestations was started in 1973. Identification of the nematode obtained from the infected roots was based upon the perineal pattern in females. The species of root-knot nematode was found to be *M. javanica*. The various new host plants found in this survey are given in Table I.

Besides the latest available literature, two comprehensive publications<sup>4,8</sup> have been consulted. As far as known to the authors, the plants given in Table I against Sr. Nos. 1, 3, 6, 8 and 10 constitute the first record of additional hosts of *M. javanica* while the remaining host plants have earlier been reported from countries other than India.

*Achyranthes aspera*<sup>6</sup> was reported as host of *M. javanica* but not the var. *prophyristachya* Hook. *Browallia* spp.<sup>5,10</sup> and *Portulaca quadrifida*<sup>9</sup> were reported as hosts of *Meloidogyne* sp. but not of *M. javanica* because species problem was first resolved and the genus *Meloidogyne* revised by Chitwood in 1949. *Cyperus rotundus*<sup>3</sup>, *Dalbergia sissoo*<sup>7</sup> and *Nicotiana plumbaginifolia*<sup>1</sup> were reported as hosts of *M. javanica*.

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#### EFFECT OF GROWTH REGULATORS ON FRUIT SETTING OF ARECANUT (*ARECA CATECHU* L.) PALMS

POOR fruit set and heavy drop in arecanut palms are serious problems affecting kernel yield adversely. Recently, many plant growth regulators have been found effective in increasing the fruit setting percentage of various fruit crops<sup>2,4</sup>. The present investigation aimed at evaluating the effectiveness of certain growth regulators on fruit set and yield of a local variety *Vittal* of arecanut, commonly grown in South Kanara District of Mysore State.

Fifty arecanut palms of ca. 15 years old, uniform in vigour and productivity were selected under this investigation. Gibberellic acid (GA), 2,4-dichlorophenoxyacetic acid (2,4-D) and N-dimethylamino succinamic acid (B-995) in various concentrations were sprayed to newly opened inflorescence. One lot kept as control, sprayed with distilled water. Altogether there were 10 treatments; each palm of single inflorescence representing a replication. Inflorescence were sprayed twice at the interval of 25 days from the first spraying.

The data on total number of female flowers per inflorescence before the sprayings, set, percentage increase or decrease over control obtained two



months after sprayings and yield at the harvest are given in Table I.

TABLE I  
Fruit setting in arecanut palms

Treatment	No. of female flowers per inflorescence	% fruit set	% increase or decrease over control	Nut yield per bunch (Kg)
Control (water spray)	219.8	23.11	..	7.0
GA 50 ppm	346.4	51.91	+124.4	11.5
GA 100 "	313.2	53.07	+129.6	18.1
GA 200 "	317.2	49.37	+113.6	15.7
2, 4-D 25 ppm	291.0	38.08	+64.7	9.4
2, 4-D 50 "	318.4	49.29	+113.2	11.4
2, 4-D 100 "	383.2	24.33	+5.2	8.5
B-995 100 ppm	364.6	45.47	+96.7	14.0
B-995 200 "	188.0	50.00	+116.3	16.8
B-995 300 "	251.4	21.24	-8.0	6.7
CD at 5% level	..	NS	..	5.3

Results showed that spraying with 100 ppm GA gave better fruit set than its other concentrations. Similarly sprayings with 50 ppm 2, 4-D and 200 ppm B-995 gave more fruit set than the other doses of these compounds but lesser than 100 ppm GA. However, the treatment differences were non-significant. Similar trends was also obtained in the case of nut yield per bunch and the treatment differences were significant. The increase in total yield of nuts was possibly due to size, weight, components of inner material and the retention of nuts at the time of harvest. In general, spraying with growth regulators caused more fruit set and kernel yield than in control, except 300 ppm B-995. Results of the present investigation are in conformity with those reported earlier in other crops<sup>1-3</sup>.

Results reported above suggest that the fruit set and yield of arecanut palms may be increased by spraying with 100 ppm (GA) or 50 ppm (2, 4-D) or 200 ppm (B-995) with two sprays.

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## DEGRADATION OF INSECTICIDES BY THE CULTURED SYMBIOTES OF *CLETUS SIGNATUS* WALKER (COREIDAE: HETEROPTERA)\*

STUDIES were conducted on the cultural, morphological biochemical, pathological and serological characteristics and the mode of transmission from one generation to another generation of the hereditary micro-organisms harboured by the bug *Cletus signatus* Walker in the mycetome situated in the last section of the mid gut. Due to their being hereditary in nature and their presence in all the individuals, such type of micro-organisms were claimed to be symbiotes (Buchner, 1965). Although their functions have been determined only in few cases, the symbiotes of *C. signatus* on the basis of various characteristics as in *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, Smith, 1957) were identified as *Bacillus cereus* var. *signatus*. In this paper, studies conducted to establish the role of symbiotic micro-organisms in degradation of various insecticides have been described. Details of other findings will be published elsewhere.

**Materials and Methods.**—One insecticide, namely, DDT, parathion and carbaryl from each of the groups, *i.e.*, chlorinated hydrocarbons, organophosphates and carbamates, respectively, were chosen for determination of degradation. Technical grades of various insecticides were used. Ten ml of nutrient broth were taken in various test tubes and autoclaved at 15 lb pressure for 15 minutes and inoculated from the pure culture of symbiotic micro-organisms and later incubated for 24 hours at 30° C to have a thick growth of micro-organisms. After this period a definite quantity of filter sterilized insecticides (Table I) in acetone solution was added. Necessary uninoculated controls were maintained. After addition of insecticides both treatment and control tubes were incubated at 30° C for different time periods (Table I) followed by extraction of insecticidal residue from broth using benzene for parathion and DDT, and methylene chloride for carbaryl and then analysis by the methods described in the Official Methods of Analysis (1970) (Assoc. of Official Analytical Chemists).

**Results and Discussion.**—It is evident from the results of the experiment summarised in Table I. that symbiotes of *C. signatus* rapidly degraded the insecticides like DDT, parathion and carbaryl, when these were incubated with cultures. Degradation was much more pronounced in 6 hours and in 24 hours of incubation in which 820 and 850 µg of DDT, 29 and 60 µg of parathion and 18 and 22 µg of carbaryl were degraded respectively. Beyond 24 hours utilization of the pesticides

TABLE I  
Bacterial degradation of insecticides

Name of insecticides	Quantity added ( $\mu$ g a.i. per tube)	Period of incubation (hours)	Replication No.	Absorbance (Mean)			Amount of insecticide degraded $\mu$ g
				Control	Treatment	Difference	
DDT	1000	6	3	0.285	0.050	2.234	820.00
	1000	24	3	0.280	0.036	0.244	850.00
	1000	72	2	0.275	0.040	0.235	820.00
Parathion	100	6	3	0.134	0.086	0.048	29.00
	100	24	3	0.130	0.043	0.087	60.00
	100	72	3	0.126	0.034	0.092	67.00
Carbaryl	50	6	3	0.372	0.239	0.133	18.00
	50	24	3	0.361	0.201	0.160	22.00
	50	72	3	0.344	0.178	0.166	22.50

was rather negligible. *B. cereus* generally starts sporulating by 24 hours and this may be the cause of the near stoppage of the metabolism of the pesticides.

The value of this characteristic of symbiotes to the host insect can be determined by the *in vivo* studies on the comparative susceptibility of the aposymbiotic and normal bugs to the insecticides. Due to failure to produce aposymbiotic bugs, such studies could not be conducted here. Mallory and Matasmura (1967) have also found such a degradation by the cultured symbiotes of *Rhagoletis pomonella* and have expressed that these may be helpful to the host insect.

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### EFFECT OF FUNGAL INFECTION IN VITAMIN C CONTENT OF APPLES

ALL the vitamins, or at least their intermediate precursors are synthesized in green plants. Vitamin deficiency diseases of man, such as scurvy, resulting from ascorbic acid deficiency, has been known for centuries. This vitamin which is essential for man and higher animals is mostly supplied by different fruits. Its biosynthesis by plants has been reviewed by many investigators<sup>1,2</sup>. The important vitamin contents of fruits are known to be largely affected by fungal invasions. It is, therefore, aimed to study the changes in the ascorbic acid contents of two varieties of apple fruits after infection by *Aspergillus niger* van Tiegh causing severe storage rot.

Healthy fruits of both the varieties were inoculated by *Aspergillus niger* van Tiegh and were kept at 26° C ( $\pm 1^\circ$  C) for a period of 12 days. On every 3rd day 5 g each of healthy and diseased pulp were taken from both the varieties. The pulp so taken was macerated by grinding with acid washed sand and 10 ml of extracting solution<sup>3</sup> and volume of the filtrate was raised to 25 ml. The amount of ascorbic acid was then estimated by the reduction of 2, 6 dichlorophenol, indophenol and measuring changes in optical density at 520 m $\mu$  at 15 and 30 seconds intervals as described by Roe<sup>4</sup>.

The results obtained are shown in Table I. The quantity of ascorbic acid in healthy and diseased fruits is the mean of three samples.

It is observed that the amount of ascorbic acid in both the varieties is more or less the same. As the incubation progressed there was a gradual decrease in ascorbic acid content in both healthy and rotted tissues of both the varieties tested. The decline was, however, much faster in diseased fruits while it was comparatively insignificant in healthy tissue. The depletion of ascorbic acid in the diseased tissue may be ascribed to its being lost

due to oxidation. In the present study the gradual decrease in the quantity of ascorbic acid in healthy fruits could be attributed to the overripening of the fruits, as also has been reported by many investigators<sup>5-7</sup>.

TABLE I

The change of ascorbic acid content in healthy and diseased fruits of two apple varieties infected by *Aspergillus niger* (mg/100 g fruit pulp)

Apple varieties		Incubation period in days				
		Zero	3rd	6th	9th	12th
KESARI	Healthy	2.08	2.00	2.00	1.8	1.7
	Diseased	..	0.791	0.426	0.00	0.00
EDWARD	Healthy	1.983	1.980	1.881	1.761	1.7061
	Diseased	..	0.356	0.1692	0.00	0.00

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#### NOCTURNAL POLLEN GERMINATION IN *IMPATIENS* (BALSAMINACEAE)

SEVERAL studies have provided evidence for an intriguing series of relationships among certain dynamic as well as static aspects of pollen grain biology. Although a good deal of data on *in vitro* as well as *in vivo* germination of pollen in day-blooming plants are available, the knowledge on adaptations of night-blooming angiosperms for nocturnal pollination is very meagre. The present work on *Impatiens* L., (Balsaminaceae) intends to study the nature and behaviour of pollen in different species occurring in South India. The taxa

of the genus are highly evolved among Geraniales as evident from their marked zygomorphy of flowers and nectiferous spur. The arrangement of stamens, pistil and spur is markedly adapted for cross pollination. However, the intricacies of reproductive biology and breeding behaviour of these plants are still imperfectly known. The present investigation on *Impatiens* has shown for the first time that the pollen grains of different species will have different germination timings, and has helped in understanding the mode of pollination in these species.

The materials for the present study were collected from high-altitude ranges of South India and cultivated in the green house. Regular observations were made for the study of phenological characters such as season of flowering, time of opening and shedding of flowers. The scapigerous species with tuberous rootstocks which did not survive in the green house, were subjected to phenological observations on the same night or two to three succeeding days and nights after they were brought alive to the green house from the nearest available localities.

Since the pollen in *Impatiens* was found to germinate readily in distilled water, this method was standardised for all the species studied except in the occasions when the pollen did not germinate during the day hours (e.g., *I. acaulis* and *I. scapiflora*). In the latter case the sucrose solution of 2.4, 5, 10% concentrations was used. The petri-dishes were covered with wet blotters to maintain cold and humidity inside. Pollen of 22 taxa was subjected to germination test, and the phenological data obtained are given in Table I.

As it is evident from Table I. a majority of species of *Impatiens* are night-blooming and have a wide range of timings with regard to pollen germination. Among the species investigated, *I. acaulis* (Naduvattam) and *I. scapiflora* (Jodpala, near Mercara) collected during the late South-West monsoon are of special interest. When the pollen grains from the opened flowers of these species were put in water or sucrose medium, they failed to germinate either under decreased or increased temperature conditions. From the phenological observations made during night it was revealed that the flowers in these species are chiefly night-blooming. Pollen from just-opened flowers in the night when put in water resulted in the maximum germination within a short duration (10 minutes). The rate of growth of pollen tube in the beginning was found to be 56.25  $\mu$  in length per minute. In *I. scapiflora* brought from Jodpala during July-August, retained viability for nearly 24 hours or even more under cool and humid environment. On the other hand,

TABLE I

Phenological characters of *Impatiens* L., of South India

Taxa	Locality	Period of flowering	Anthesis	Pollen germination
St.: SCAPIGERAE				
1. <i>I. acaulis</i> Arn.	Naduvattam	July–Sept.	Nocturnal	Nocturnal
2. <i>I. acaulis</i> var. <i>granulata</i>	Agumbe	"	"	Nocturnal and following day
3. <i>I. scapiflora</i> Heyne	Jodpala	"	"	Nocturnal
4. <i>I. modesta</i> W.	Naduvattam	July–Oct.	"	A few only germinate in night and day
5. <i>I. barberi</i> Hk.f.	Bisle Ghat	August–Oct.	Diurnal	Uncertain
St.: ANNUAE				
6. <i>I. chinensis</i> L.	Hassan, Coorg	July–Nov.	"	No specific time
7. <i>I. pusilla</i> H. ync. var. <i>inconspicua</i>	Naduvattam	"	"	"
8. <i>I. kleinii</i> W. & A.	Subramanya	July–Oct.	"	Diurnal and Nocturnal
9. <i>I. tenella</i> Heyne	Naduvattam	"	"	"
10. <i>I. gardneriana</i> W.	Palghat	July–Nov.	Nocturnal	"
St.: MICROSEPALAE				
11. <i>I. leschenaultii</i> Wall.	Nilgiris	July–Dec.	Diurnal and nocturnal	Diurnal
12. <i>I. talpobi</i> Hk.f.	Agumbe	"	Nocturnal	Nocturnal
13. <i>I. balsamina</i> L. (wild)	Chamundi Hills	Oct.–Dec.	"	Nocturnal and Diurnal
14. <i>I. scabriuscula</i> Heyne	Naduvattam	July–Nov.	"	No specific time
15. <i>I. flaccida</i> Arn.	Bababudans	"	Diurnal	"
16. <i>I. repens</i> Moon.	Native of Ceylon	July–Oct.	"	"
St.: SUB-UMBELLATAE				
17. <i>I. hookeriana</i> Arn.	Nilgiris	Oct.–Jan.	Nocturnal	"
18. <i>I. fruticosa</i> DC.	Naduvattam	August–Nov.	Diurnal	"
19. <i>I. sultanii</i> Hk.f., var. red	Cultivated	Throughout the year	Midnight	Diurnal
20. <i>I. sultanii</i> Hk.f., var. white				No specific time
21. <i>I. campanulata</i> Wt.	Devikolam, Kodaikanal	Dec.–Feb.	Nocturnal and Diurnal	Uncertain
St.: EPIPHYTICAE				
22. <i>I. parasitica</i> Bedd.	Devikolam	Oct.–March	Morning	Diurnal

*I. acaulis* the pollen grains retained viability only first few hours following anthesis. The percentage of germination and length of pollen tube come progressively reduced; and during next day only a few pollen germinated, if at all. Further studies indicated in *I. acaulis* and *scapiflora* that there is a self-incompatible system which has been brought by non-synchrony in the time of embryo sac and pollen maturation. Although the maturation of the pollen takes place earlier than embryo sac, liberation of pollen occurs only after the opening of the flowers in the night, and a female gametophyte at that time would have hardly reached 2–4 nucleate stage and thus is not mature enough for getting self pollinated. The flowers remain open for most parts of the following day retaining the stamens and during which a female gametophyte attains full maturity. But to ensure any fertilisation, there will not be enough available viable pollen and thus the female gametophyte is made to await for the freshly available pollen in the following night. As the pollen retains viability for only 2–3 hours after anthesis (e.g., *I. acaulis*), the

female gametophyte will have to be pollinated only during this short duration.

Thus in addition to their characteristic features like predominant white colour, long tubular corolla or spur, and exuberant fragrance (Bhaskar, 1972), night-blooming flowers are found to have a remarkable adaptation as to their pollen germination also. One plausible explanation for the occurrence of nocturnal anthesis and pollen germination is that during the course of time a change from a more moist and cool climate in the atmosphere to a drier climate (Menon, 1968; Seth, 1962) or any such change in the climate (Rege *et al.*, 1959) might have induced some of the species to adapt to coolness of the night. In case of other night-blooming species like *I. gardneriana*, *I. balsamina*, *I. hookeriana* and *I. sultanii*, they have not only retained their pollen viability for sufficiently longer period (a day or two), but also their flowers remain open long after daybreak, so that day pollinators complete the process in case pollination has failed or remained incomplete during the previous night. This condition appears to be more advantageous

as it helps in the preservation of a species, particularly under such severe climatic conditions as are found at high altitudes. The differences in the time of anthesis and pollen germination in these highland species may relate to such factors as the day length, temperature and humidity; however, further experimental work is needed to solve the mysteries behind this remarkable adaptation of night blooming species.

One of the authors (V. B.) was supported by C.S.I.R., New Delhi, India.

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#### NITROGEN FIXATION BY A NON-SPORULATING MUTANT STRAIN OF *ANABAENA DOLIOLUM* BHARADWAJA

MORE than 50 strains and species of heterocystous algae are known to fix atmospheric nitrogen<sup>1</sup>. The contribution of phototrophic blue-green algae to the nitrogen status of natural ecosystems is probably much more important than that of heterotrophic micro-organisms. In view of the meagre work on the estimation of growth and nitrogen fixation by mutant strains of blue-green algae of rice fields, the present work was undertaken to study the growth and amounts of nitrogen fixed by a non-sporulating mutant strain of *Anabaena doliolum* Bharadwaja, isolated from paddy fields and maintained in our laboratory. This alga forms both terminal and intercalary heterocysts and every vegetative cell in the parent strain gets transformed ultimately into a spore.

The alga was first made bacteria-free by ultraviolet irradiation. The purity of the culture was tested in a bacteriological medium containing 0.2% yeast extract, 0.2% bacteriological peptone and 0.5% dextrose. *A. doliolum* was grown in conical flasks, each containing 100 ml Allen and Arnon's (nitrate-free) medium<sup>2</sup>, for a period of 50 days and its growth and amounts of nitrogen fixed were determined every 10-day intervals. All the replicate flasks were incubated under light intensity of two 60 watt tungsten lamps at a temperature of  $28 \pm 2^\circ \text{C}$ . The growth of the alga was measured on dry weight basis. The nitrogen contents of alga and culture filtrates were determined by micro-Kjeldahl method, using a selenate catalyst mixture in the digestion.

Table I shows the dry weights and nitrogen contents of *A. doliolum* at 10-day intervals. Statistical analysis revealed a strong correlation between growth and percentage of total nitrogen fixed ( $r = 0.9002$ ; significant at 5% level). The regression equation obtained was per cent total  $N = 3.06 + 0.111 \times \text{dry wt}$ . The growth and corresponding total nitrogen content of the alga increased till the end of the experiment. The increase in nitrogen in the growth medium represented the atmospheric nitrogen fixed and excreted by the alga.

TABLE I  
Growth and nitrogen contents of a nonsporulating strain of *A. doliolum*

Growth (days)	Dry weight of alga (mg)	N content of alga		N content of culture filtrate (mg)	Total N	
		(mg)	(%)		(mg)	(%)
10	10.1	0.350	3.46	0.200	0.550	5.44
	12.4	0.412	3.32	0.128	0.540	4.35
	(12.6)		(2.83)			(4.15)
20	15.3	0.264	1.72	0.145	0.409	2.67
	14.2	0.625	4.40	0.129	0.754	5.30
	18.0	0.602	3.34	0.231	0.833	4.62
30	(14.9)		(4.00)			(4.73)
	12.5	0.534	4.27	0.150	0.684	4.27
	20.0	0.721	3.60	0.238	0.959	4.79
40	15.0	0.602	4.01	0.231	0.833	5.55
	(16.0)		(3.74)			(5.22)
	13.0	0.469	3.60	0.224	0.693	5.33
50	22.0	0.868	3.94	0.490	1.350	6.13
	21.0	0.707	3.36	0.350	1.057	5.03
	(20.8)		(3.67)			(5.46)
50	19.4	0.721	3.71	0.294	1.015	5.23
	28.0	1.029	3.67	0.357	1.386	4.95
	27.0	0.889	3.29	0.567	1.456	5.39
50	(24.3)		(3.80)			(5.59)
	18.0	0.778	4.43	0.364	1.162	6.45

The tropical paddy fields offer a variety of diverse habitat conditions such as water-logging, high temperature and high humidity which are conducive to luxuriant growth of many algae. According to one estimate<sup>3</sup> about 75% of the algae in Indian paddy soils may be blue-green algae where they develop profusely with photosynthetic bacteria. The nitrogen which these algae fix during the growth is excreted into the soil in the form of peptides, free amino acids and other nitrogenous compounds<sup>4-7</sup> which becomes subsequently available to the associated rice plants.

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# AN EDIBLE ALGA OF MANIPUR (*LEMANEA AUSTRALIS*): PRESENCE OF SILVER

DEB<sup>1-5</sup> collected an interesting alga in Manipur State, locally called *Nungsam* which is sold as an article of food. It is a freshwater Rhodophyta representing the family Lemnaceae and is determined as *Lemanea australis* Atkins<sup>6-8</sup>. It is found on the rocks in the swiftly flowing fresh water at the confluence of the Manipur and Chakpai rivers (24° 16' 35" : 93° 52' 30", Topo sheet 83 H/SE) at a distance of about 1.6 km south of Shugnu in Manipur State. The plant grows luxuriantly but for few months in the early winter, when it is collected and dried. In Manipur it is cooked with vegetable primarily for its characteristic fishy smell. No reference could be traced from literature<sup>9-15</sup> in this regard. This is, however, reminiscent of the uses of *Enteromorpha intestinalis* Link<sup>16-18</sup>. The sample on analysis (dry wt. basis) showed 20% protein, 32.5% carbohydrate and 10% lipid.

No toxic effect is reported as yet, although it is eaten by local people for generations. Biological evaluation of the material in animals should be carried out to specify nutritive value of protein, etc.

Spectrographical analysis of ash conducted in the Chemical Laboratory of Geological Survey of India, Calcutta, reveals the presence of trace elements as shown in Table I.

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TABLE I

Element	Cu	Pb	Zn	Ni	Co	V	Mn	Ag	Ba	Sr	Cr	Zr
Quantity ppm	>200	100	1600	100	60	20	>3000	4	200	100	100	40

Of the trace elements the most interesting is the presence of silver which has not been reported from any Indian plant, nor is there any report on the occurrence of this mineral in the area in which this plant is found.

The use of plants as an indicator of economic deposits has been recently summarised by Cannon<sup>22</sup>. He did not mention silver which, however, has been detected in several plants<sup>23-27</sup>. Presence of silver as a trace element in the plant indicates its existence in the rock on which it grows. A thorough survey of the region is desirable to explore the potentiality of the rocks involved.

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## SHORT SCIENTIFIC NOTES

### A New Fruit Rot of *Zizyphus jujuba* Lamk.

During the course of surveys for the fungal diseases of certain fruits and vegetables of Rajasthan, the authors observed a severe fruit rot of *Zizyphus jujuba* in the local fruit market of Bharatpur in January, 1973.

In the early stages the diseased fruits showed small, dark-brown spots. In later stages the spots enlarged and became dark ash-gray coloured. Finally infected fruits got slightly depressed and many fruiting pustules were produced on these spots.

On examination, the fruiting pustules were found to be ascervuli of the *Pestalotia versicolor* Speg.

Pathogenicity of the organism was confirmed by inoculating the fruits by Granger and Horne's<sup>1</sup> method and also by spraying the conidial suspension of the organism over the injured and uninjured fruits. Only injured fruits developed typical symptoms. The fungus was reisolated from these artificially inoculated fruits and was found to be similar with the original isolate.

This is the first report of *Pestalotia* rot of *Zizyphus jujuba* from India.

The authors are thankful to Professor H. C. Arya for providing laboratory facilities. Thanks are also due to Dr. D. K. Purohit for identification of the fungus.

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### Leaf Spot of *Prunus tomentosa* Caused by *Cladosporium herbarum*

In May 1972 the leaves of *Prunus tomentosa* plants growing at Horticultural Research Station, Simla, to study its potentiality for using as root stock for peach and other stone fruit plants exhibited the leaf spot symptoms.

The primary infection of the disease on the foliage was noticed as minute, oval to irregular, light brown to greyish white with purple spots measuring 1 to 6 mm in length and 1 to 3 mm in breadth. The infections started from the margin and gradually moved down to the base of the leaf blade. The minute spots enlarged and coalesced forming bigger

patches. In the advanced stage of disease development these spots covered the entire leaf and developed blighted areas on the surface of the leaf lamina, which turned necrotic and drop out leaving 'shot holes'.

The fungus was isolated on PDA and identified as *Cladosporium herbarum* (Pers.) Link ex S.F. Gray and its identity was confirmed by CMI, Kew, Surrey, England. The culture of the pathogen as well as leaf specimens have been deposited at CMI under succession No. IMI 173564.

Pathogenicity test of *Cladosporium herbarum* on the newly formed leaves were also studied. *Prunus tomentosa* plants in pots were inoculated with the suspension of 7 days old culture of pathogen consisting of both mycelium and spores. The inoculated plants were covered with moist polythene bags for 48 hours. The uninoculated plants were maintained as control. The minute purple necrotic spots developed after 7 days of inoculation. The control plants did not show any symptoms. Reisolation from infected parts yielded the same pathogen which was similar to original isolate.

*Cladosporium herbarum* (Pers.) Link ex S. F. Gray, occurring on *Prunus tomentosa* has shown to be first record from India. Similar fungus has been recorded on citrus<sup>1</sup> and *Prunus persica*<sup>2</sup> from India.

The authors wish to express grateful thanks to Dr. S. P. Raychaudhuri, Head, Division of Mycology and Plant Pathology and to Dr. R. N. Singh, Head, Division of Horticulture and Fruit Technology, I.A.R.I., New Delhi, for the encouragement.

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### A New Host Record for *Erysiphe polygoni*

During the month of September–October 1973, a weed plant *Tephrosia purpurea* Pers. was observed to be infected with powdery mildew. The mildew attacked the lower leaves and later developed on younger leaves, covering the entire leaf surface with the dense coating of powdery mass, Till

December only the conidial stage of the pathogen was observed. In the month of January brown to black coloured small dot-like perithecia, scattered on both the surfaces of the leaves were observed. The perithecia continued to develop up to March till the plant drops its leaves, however, newly formed leaves in the month of May, were healthy.

The mildew fungus was found to have hyaline mycelium. The perithecia were scattered, brown to dark brown in colour, superficial, almost globose with  $108.10-169.50$  ( $143.83 \mu$ ) in diameter, bearing myceloid appendages and containing 3-8 broadly ovate, slightly stalked to sessile asci measuring  $39.95-61.10 \times 25.85-42.30$  ( $50.14 \times 34.43 \mu$ ). Each ascus contained 3-5 (more frequently 4), oval to oblong, one-celled ascospores of  $13.90-25.85 \times 9.40-16.45$  ( $18.30 \times 12.35 \mu$ ) size. On the basis of morphology and measurements of perithecia, asci and ascospores the mildew fungus has been identified as *Erysiphe polygoni* DC.

In the literature so far available, there seems to be no previous report of the occurrence of *E. polygoni* on this weed. Therefore, the present note records *Tephrosia purpurea* Pers. as a new host for *E. polygoni*.

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**Crab Caterpillar—*Stauropus alternus* Wik. (Notodendridae : Lepidoptera), A New Pest of Sapota (*Achras sapota* L.)**

*Stauropus alternus* Wik. has been mentioned as occasional and minor pest of red gram (*Cajanus indicus* L.), tamarind (*Tamarix indica* L.), tea [*Camellia sinensis* (L.) O. Kuntze], *Mangifera indica* L., *Mangifera* sp. *Theobroma cacao* L., *Xylia dalberiformis* B (= *X. xylocarpa* T.) and *Terminalia paniculata* (Ayyar, 1960<sup>1</sup>; Mathur and Singh, 1959 and 1960)<sup>2,3</sup>. In October, 1973, larvae of *S. alternus* were found causing serious damage to the foliage of newly planted sapota grafts at Regional Research Station, Mudigere. The insect is placed on record for the first time as a pest of sapota and some observations made on the biology and damage of the pest are reported here.

The adult moth lays 98-100 whitish-blue eggs, singly on the undersurface of the leaf close to the margin. The newly hatched caterpillars are blackish to brown, elongate with long appendages and are found in pairs in the leaf axils. Larvae feed gregariously on the leaves during daytime from the margins to the midribs almost symmetrically from either sides. In case of severe damage, the whole leaf is eaten leaving behind only the midrib.

The damage is distinguished by the presence of excretory pellets and cut leaf bits in the drip-line. The full grown caterpillars measure 5.5 cm in length. Pupation takes place on the leaves webbed in a silken cocoon.

The authors are grateful to Prof. S. D. Kololgi, Chief Scientific Officer (Hort), for encouragement.

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**New Records of Alternative Host Plants of Red Cotton Bug, *Dysdercus cingulatus* Fab.**

The red cotton bug (*Dysdercus cingulatus* Fab.) is considered to be one of the major pests of cotton (Sohi, 1964)<sup>1</sup>. Its attack has been noticed on bhindi (*Hibiscus esculentus*), hollyhock (*Althaea rosea*), Deccan hemp (*Hibiscus cannabinus*), musk mallow (*Hibiscus abelmoschus*), shoe flower (*Hibiscus rosasinensis*), silk cotton (*Bombax malabaricum*), portia tree (*Thespesia populnea*), maize (*Zea mays*), bajra (*Pennisetum typhoideum*), wheat (*Triticum aestivum*), kossum (*Schleichera oleosa*), cape gooseberry (*Physalis peruviana*), (*Solanum verbascifolium*), etc., by various workers in India.

During November 1972, the nymphs of red cotton bug were found feeding on wild castor (*Chrozophora rotleri*, Fam. Euphorbiaceae) and kanchamanda (*Trichodesma amplexicaule*, Fam. Boraginaceae), weed plants in the fields of Punjabrao Krishi Vidyapeeth, Akola (Maharashtra).

In order to ensure that wild castor and kanchamanda would support the bugs, the nymphs and adults were collected and brought to the laboratory, and provided with weed twigs bearing fruiting structures. The cut portion of twigs was dipped in water in a glass vial to keep the food fresh. Both the nymphs and adults were kept in wire-gauze cages along with the food. The food was changed thrice a week.

It was observed that bugs actively continued feeding and majority of them survived throughout winter. Nymphs moulted normally. A few of the mated females deposited eggs. The eggs hatched and the newly hatched nymphs fed well on the twigs of wild castor and kanchamanda weeds to become adults.



This is a new record of alternative host plants of red cotton bug in India and these weeds serve as good food plants for overwintering adults.

The authors are grateful to Dr. K. R. Thakare, Head of the Department, for providing necessary laboratory facilities, and to Dr. S. B. Lall, Professor of Agricultural Botany, College of Agriculture, Nagpur, for identifying the host plants.

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#### Occurrence and Control of the Stunt Nematode, *Tylenchorhynchus brassicae* Siddiqi, 1961 Infesting Rice Nursery in Punjab

The association of *Tylenchorhynchus brassicae* is known to cause damping off of seedlings, general stunting and poor growth of cauliflower (Khan *et al.*, 1971). In Punjab, at the Regional Rice Research Station, Kapurthala, *T. brassicae* was found occurring for the first time in 1973 in paddy. On an average 387 nematodes per 250 cc of soil sample were recorded. Since it is associated with the stunting of crops, a trial was laid out with granular nematicides for its control in the rice nursery.

Carbofuran (Furadan 3 G) at 1.0 and 1.5 kg, fensulfiothion (Dasanit 5 G) at 2.5 and 5.0 kg, aldicarb (Temik 10 G) at 2 and 3 kg and phorate (Thimet 10 G) at 2.5 and 5.0 kg a.i./ha were broadcast and raked in the plots each measuring 2 m<sup>2</sup>, about a week after sowing nursery. Immediately after the granular applications, a light shower irrigation was given. Each treatment including control was replicated thrice. The nematode population counts were made from a composite soil sample of 250 cc from each plot immediately before and 20 days after treatment.

The data furnished in the following table reveal that the pre-treatment nematode population was non-significant. However, 20 days after treatment, carbofuran 1.5 kg, fensulfiothion 5 kg and aldicarb 3 kg were found to be equally and highly effective in controlling the nematode. While fensulfiothion 2.5 kg, carbofuran 1 kg and phorate 5 kg followed in efficacy and were at par among themselves, Aldicarb 2 kg and phorate 2.5 kg were only as good as control.

TABLE I

Control of *Tylenchorhynchus brassicae* Siddiqi, 1961 in rice nursery in Punjab

Treatment	Dosage kg a.i./ha*	Nematode population counts per 250 cc soil sample (Average of three replications)	
		Pre- treatment	Post- treatment
Carbofuran (Furadan 3 G)	1.0	440	197 (13.77) b†
"	1.5	377	17 (4.02) a
Fensulfiothion (Dasanit 5 G)	2.5	423	157 (12.39) b
"	5.0	247	20 (4.37) a
Aldicarb (Temik 10 G)	2.0	473	323 (17.70) bc
"	3.0	350	27 (5.09) a
Phorate (Thimet 10 G)	2.5	393	380 (19.40) c
"	5.0	433	207 (13.78) b
Control	..	350	433 (20.71) c
C.D.=(p=0.05)		NS	(5.12)

\* Kg active ingredient per hectare.

† Figures in parentheses are  $\sqrt{n}$  transformations.

For the control of *Tylenchorhynchus claytoni* infesting a potato field, 10% granules of aldicarb and carbofuran at 44.8 kg/ha proved highly effective whereas fensulfiothion at this dosage repelled the nematode (Miller and Kring, 1970). Temik 10 G and Furadan 5 G at 25–50 lb/acre gave high level control of citrus nematode, *Tylenchulus semipenetrans* (Baines and Small, 1969). In the present studies, carbofuran 1.5 kg, fensulfiothion 5 kg and aldicarb 3 kg a.i./ha were highly effective in controlling *T. brassicae*, which is in conformity with the previous findings.

The authors thank Dr. O. S. Bindra and Dr. K. S. Gill for providing facilities and to Dr. R. Mahajan for identifying the nematode.

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## REVIEWS AND NOTICES OF BOOKS

**Basic Electrotechnology.** By H. Cotton Macmillan. (The Macmillan Press Ltd., London), 1973. Pp. 306. Price £2.50.

Professor Cotton is quite familiar to the students of electrical engineering for more than four decades through his distinguished text-books. In the words of the author, this latest book has been written "to cover the syllabus of the subject of Electrical Principles II of the City and Guilds London Institute". International system of units, or SI units for short, has been used throughout.

The book under review presents the fundamental concepts of electrical engineering principles. Starting with an introductory chapter on SI units, the author proceeds to develop the topics of electricity, potential, electric current, heat, power and energy. Elementary electric networks and electromagnetism are then explained. Principles of alternating currents, construction of electric machines and their windings have been dealt with. The author also devotes a few chapters to cover topics like electronic devices, electrical and electronic measuring instruments.

This volume which serves as a useful introduction to the subject, is well written and produced. This is also adequately illustrated. Numerical examples are given throughout the text. A set of problems with answers, arranged chapterwise, is given at the end.

This book can be recommended to the students and engineers requiring a first introduction to the principles of electrotechnology.

The only point not in favour of this book is its price, which is on the higher side. K. S. PRABHU.

**Linear Methods of Applied Analysis.** By Allan M. Krall. (Addison-Wesley Pub. Co., Advanced Book Programme, Reading, Massachusetts), 1973. Pp. xiv + 706. Price: Cloth binding \$16.00; Paper binding \$9.50.

This book is meant to be a text-book serving an introduction to Applied Mathematics for upper undergraduate and beginning of master's level courses in Mathematics, Engineering and the Physical Sciences. The book is a good addition and deals with the topics of Vector spaces, matrices, Hilbert spaces, linear operators and ordinary and partial differential equations. The contraction mapping theorem, existence and uniqueness theorem

for ordinary differential equations, Stone-Weierstrass theorem, special functions, Fourier integral, the singular Sturm-Liouville problem, and distribution theory are introduced at proper places of the development of the book and are used in due course. The author attempts to keep the treatment modern mathematical and theoretically oriented, the applications being mentioned for motivation. For detailed development of the applications one should consult other books.

The discussion of partial differential equations is not as general as one would expect from the tempo of the treatment in the earlier part of the book. As it is mentioned at the bottom of page 473, and on page 482, the setting of discussion is  $E^n$ . At some moments the author does not mind keeping rigour away from the discussions as he mentions at the end of page 464. In such situations, one would be required to resort to other better text-books for applied mathematics students.

Some examples of the imprecise statements in the book are the following. In line 14 of page 6, the words 'greater than or equal to' should have been used. In the definition of determinant the terms 'Cyclic' and 'Acyclic' should be replaced by the terms 'even' and 'odd' respectively on page 93. For example, the permutation (2, 3, 1, 4, 5) is not a cyclic, but an even permutation of (1, 2, 3, 4, 5). The example in first para on page 102 is not satisfactory. It is not clear how  $\phi$  and  $\eta$  are independent.

Reviewer feels that the author should have chosen to define linear space over a general field rather than over the field of complex numbers (page 17).

$\sum a_i b_i$  should be replaced by  $\sum a_i \bar{b}_i$  in line 7 of page 6. In the last two lines of page 168,  $|b|^2$  should appear in place of  $b^2$ . In lines 4 and 7 on page 171, it would add to clarity if it is mentioned that the elements belong to  $H$  rather than to  $X$ .

Some examples of misprints are the following. On page 92, the last line of the proof of the theorem should be  $AX$  and not  $Ax$ . On page 168, right hand bracket is missing for  $f(x, y)$  in line 6. On the top of page 217, 'VIII.3' should replace 'VII.3'. In the seventh line on page 307, the word 'these' is misspelt.

Reviewer welcomes the book on the whole and recommends to bring it out in low-cost edition.

V. G. TIKEKAR.

**Kavaka**—*A New Journal of the Mycological Society of India.* (The Mycological Society of India, Centre of Advanced Studies in Botany, University of Madras, Madras-5), 1973. The Annual Subscription for members is Rs. 25.00 in India and £2.00 or U.S. \$6.00 in the soft and hard currency areas respectively. Annual subscription for Institutions is Rs. 50.00; in India and £4.00 or U.S. \$12.00 for soft and hard currency areas respectively. Subscriptions are to be sent to the Treasurer, Dr. K. G. Mukerji, Department of Botany, University of Delhi, Delhi.

In these days of specialisation, it is a happy augury that the Mycological Society of India founded in January, 1973 has started a journal with an apt Sanskrit name **KAVAKA** which means a Fungus. The emblem of the journal which has the proportions of a Golden Rectangle has meaningful objectives which are explained on the inner cover page and it is most gratifying that the first volume with 1-4 numbers for the year 1973 has recently been published under the editorship of Professor C. V. Subramanian, a well-known authority in the field of Fungi Imperfecti. This volume, which runs to 103 pages deals with various phases of Mycology including the Hyphomycetes, Phycomycetes, Basidiomycetes, Ascomycetes, Lichens and other allied disciplines. The 17 articles included in this volume are contributed from well-known workers in the field of Mycology both from India and abroad covering various aspects of fungi like taxonomy and systematics physiology, life-cycles, sporulation and experimental studies. It will be thus seen that a wide spectrum of subjects in the field of Mycology have been covered. Hence, this journal will be of particular use to all mycologists since the study of the fungi from time immemorial has great economic significance. The printing and the get-up of the journal is excellent and it is our sincere wish that it will be widely consulted all over the world, in scientific and educational institutions, where Mycology is studied.

K. SUBRAMANYAM.

## ANNOUNCEMENTS

### Nuclear Physics and Solid State Physics Symposium (1974)

The Symposium will be held at Bhabha Atomic Research Centre, Trombay, Bombay, from 27th to 31st December 1974. Further information may be had from: Dr. K. R. Rao, Convener, Nuclear Physics and Solid State Physics, Symposium Committee, Nuclear Physics Division, Modular Laboratories, Bhabha Atomic Research Centre, Trombay, Bombay 400085.

### Award of Research Degree

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Chemistry to Shri Rama Chandra Acharya for his thesis entitled "Structure Reactivity Correlation".

Osmania University, Hyderabad-7, has awarded the Ph.D. degree in Engineering to Shri Chenna Reddy Harnatha Reddy for his thesis entitled "New Synthesis Procedures for Two-Port Passive RC and Active RC Networks"; Ph.D. degree in Geology to Shri Mir Aneesuddin for his thesis entitled "Studies on Some Indigenous Minerals and Allied Materials for Their Utilization Filteraids"; Ph.D. degree in Geology to Shri Mir Yousuf Kamal for his thesis entitled "Sedimentology and Sedimentary Tectonics of the Kurnool System near Kurnool".

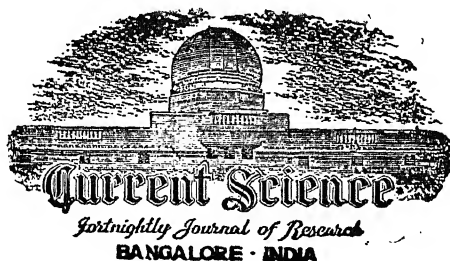
The M.S. University of Baroda, has awarded the Ph.D. degree in Medicine to Shri Anil Ranchhodji Mehta for his thesis entitled "Studies on the Effect of Maternal Protein Malnutrition on the Growth and Nutritional Status of the Foetus and Neonatal Infants"; Ph.D. degree in Medicine to Shri N. Sivaramakrishna for his thesis entitled "Uptake of Noradrenaline Tissues".

Karnatak University, Dharwar, has awarded the Ph.D. degree in Chemistry to Shri Sirmokadam Narayana Narasingarao for his thesis entitled "Spectroscopic Studies of Lead (IV) and Thorium (IV) Complexes"; Ph.D. degree in Chemistry to Shri Patil Shankargouda Virupaxgouda for his thesis entitled "Study of Metal Complexes with 1-Nitroso-2-Naphthol and Their Analytical Application"; Ph.D. degree in Zoology to Shri Kallapur Vasudeo Laxmanrao for his thesis entitled "Histophysiological Studies of Insect Thoracic Muscles, with Special Reference to Their Energetics".

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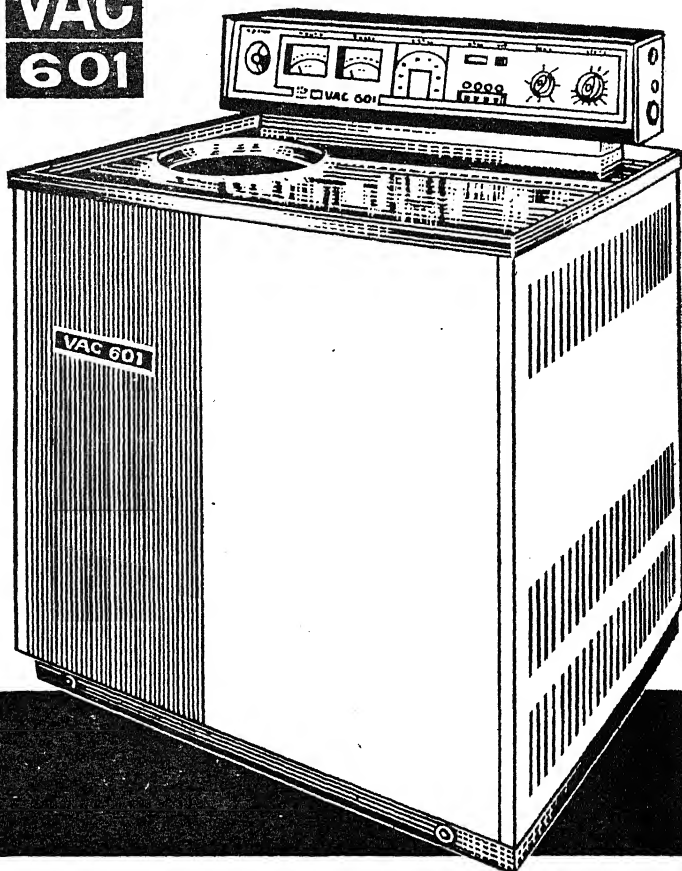
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JAISONS

## BANDING TECHNIQUES AND PLANT CHROMOSOMES

N. P. SARMA AND S. L. TANDON

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THE introduction of banding techniques in chromosome studies has made two significant contributions, (i) individual chromosomes or even part of a chromosome can now be precisely identified, (ii) these techniques have opened altogether new avenues of research on structure and molecular organisation of chromosomes. A breakthrough in the development of the technique of banding chromosomes came from the pioneering studies of Caspersson *et al.*<sup>1</sup>, that fluorescent dyes such as Quinacrine Mustard (QM) effect selective, discrete fluorescent labelling in both plant and animal chromosomes. The binding specificity of these fluorochromes and intense or reduced fluorescence at localised regions of chromosomes giving a banded appearance is known as fluorescence banding or more specifically Quinacrine banding (Q-banding; Fig. 1). The other banding technique that employs Giemsa stain is the outcome of the studies made by Pardue and Gall<sup>2</sup>, who observed denser centromeric regions of chromosomes of mouse complementary after *in situ* hybridization with complementary RNA of the mouse satellite DNA and subsequent Giemsa staining. Following this observation, a staining method with Giemsa was developed for the detection of repetitive DNA which is richly localised in the centric regions of the chromosomes<sup>3,4</sup>. The method consists of denaturation of chromosomal DNA of the metaphase preparations by heat or alkali treatment, reannealing it in a suitable buffer, followed by Giemsa staining (Fig. 2). The specific staining of centromeric regions of the chromosomes this way is called as C-banding (staining centromeric type of constitutive heterochromatin). Soon it became apparent that by employing a number of modifications it is possible to obtain differential staining of the constitutive heterochromatin in the chromosome arms (G-banding<sup>5</sup>). Through minor changes in the basic theme of denaturation and incubation prior to staining and use of proteolytic enzymes such as trypsin, many simplified techniques have been developed mostly for mammalian and human metaphase chromosomes<sup>6-17</sup>. This led to the proliferation of nomenclature of the banding techniques, viz., R-banding<sup>12</sup> (reverse Giemsa banding), BSG technique<sup>13</sup> (barium hydroxide/saline/Giemsa), N-banding<sup>16</sup> (nucleolus organiser), cd staining<sup>17</sup> (centromeric dot) and in plants pericentric banding<sup>18</sup> and Hy-banding<sup>19</sup>.

Though the initial report on banding with QM was from *Vicia faba*, the subsequent major developments in the methodology of the staining techniques stem from the experiments with the mammalian chromosomes. The ease with which the mammalian metaphase chromosome preparations can be made seems to be the primary reason for this success. Progress in this line of investigation in plant chromosomes is limited. The use of fluorescence for the detection of heterochromatic regions of the chromosomes was attempted in several plants<sup>20-25</sup>. For example, Vosa<sup>20</sup> has shown that there are several types of heterochromatin in plants as defined by allocyclic behaviour and these can be distinguished by their negative or positive sensitivity to cold and by their response to fluorochrome staining. A Giemsa staining technique is outlined by Vosa and Marchi<sup>21</sup> who compared the Giemsa banding with the pattern produced by Quinacrine



FIGS. 1-2. Fig. 1. Somatic chromosomes of *Scilla sibirica* stained with Quinacrine Mustard (Q-banding). The heterochromatic segments correspond to the regions of dull fluorescence (-). Fig. 2. Same, but stained with Giemsa. The deeply stained regions correspond to the regions of reduced fluorescence with Q-banding.

fluorescence in a number of plants with fairly large chromosomes. In all the cases, they observed a close correspondence between the regions darkly stained with Giemsa and those differentiated with Quinacrine. However, the important difference found was that the Giemsa staining does not discriminate between regions with intense and reduced fluorescence with Quinacrine, but stains both in the same way. Schweizer<sup>26</sup> developed a suitable procedure of Giemsa staining for plant chromosomes with a view to study the extent to which Giemsa bands could be correlated with heterochromatic (H)-regions revealed by cold treatment in *Trillium grandiflorum*, *Scilla sibirica*, *Vicia faba*, *Crepis capillaris* and three species of *Fritillaria*. The chromosome segments that stained strongly with Giemsa were shown to be identical with H-regions revealed by cold treatment in all the species studied. In *Crepis capillaris*, he observed, that Giemsa technique to be more sensitive than Quinacrine fluorescence in revealing the longitudinal differentiation of chromosomes. That there is a close correspondence between the regions of the chromosomes that stain darkly with Giemsa or take up bright or dull fluorescence to the heterochromatin was evident in all these investigations. However, this was questioned in the light of findings of G-banding in mammalian chromosomes<sup>6-9</sup> and also in a specific instance in plants<sup>27</sup>.

The specific banding pattern produced by these techniques are of immense value in (i) identifying the individual chromosomes as has been shown in *Petunia hybrida*<sup>28</sup>, rye<sup>29-31</sup> and *Triticale*<sup>32</sup>, (ii) the detection of structural changes in reconstructed karyotypes of *Vicia faba*<sup>33</sup> and (iii) studies in chromosome polymorphism in plant populations as reported in *Scilla sibirica*<sup>22</sup>. There are two illustrious examples to show how these banding techniques could be of specific use in plant cytogenetical investigations. Sarma and Natarajan<sup>20</sup>, using a new fluorochrome compound, *bis*-Benzimidazole derivative (Hoechst 33258) characterised the individual chromosomes of rye. The localisation of heterochromatin in the telomeres of the rye complement and its absence at the centromeric regions, as observed by these staining techniques, made possible the identification of chromosome complement of rye in the *Triticale*. Similarly, Natarajan and Sarma (in press) made a complete genome analysis in the hexaploid wheat, *Triticum aestivum* ( $2n = 6x = 42$ ) using Giemsa banding pattern. Of the three genomes of wheat A, B and D, the source of B genome is controversial. The different genomes of hexaploid wheat and its close relatives are composed of chromosomes with median and sub-median centromeres which offer no distinctive features of

morphology to identifying them<sup>34</sup>. Giemsa banding of these wheat chromosomes unravelled some morphological details, unobtainable through conventional staining. Some of the chromosomes of the wheat complement showed large, distinct darkly stained regions with Giemsa, located proximal to the centromere. The other chromosomes, however, do not possess such large blocks of centromeric heterochromatin but have heterochromatic regions in the form of bands either sparsely distributed or dispersed at the interstitial and terminal regions of the chromosomes. With such details gathered from the Giemsa banding pattern in wheat, it was possible for them to throw more light on the chromosome characteristics of the B genome. In view of the recent divergence of opinion regarding the validity of *Aegilops speltoides* being the B genome donor<sup>35-36</sup>, such a study might help in tracing the B genome donor species.

Giemsa staining technique is also used to specifically stain the telomeres of *Allium cepa* chromosomes, both at metaphase and interphase<sup>37</sup> and in the study of karyotypic differences in some species of *Anemone* and *Hepatica nobilis*<sup>38</sup>. Some modifications in the basic procedure of banding were suggested in plant chromosomes. These include use of acetic-orcein in the place of Giemsa<sup>39</sup>, digestion with trypsin prior to Giemsa staining<sup>40</sup>. More recently, Schweizer<sup>41</sup> reported an improved Giemsa C-banding procedure for plant chromosomes. These are some examples where banding techniques were applied in plant chromosome studies. However, in comparison to the literature on this subject in mammalian cytogenetics, they are rather few and limited. The practical difficulties in obtaining cytoplasm free metaphase spreads of plant chromosomes seem to be the main hindrance for the progress. Conventional acid hydrolysis used for this purpose interferes with the production of bands either with Giemsa or fluorochromes. The use of enzymes, snail gut cytase<sup>42</sup> and cellulase<sup>43</sup> for squash preparations of plant chromosomes for fluorescent studies is suggested. Mild hydrolysis with 0.1 N HCl for a few seconds or pretreatment of fixed root tips in 45% acetic acid may also help in softening the tissue. Nevertheless, there is an imperative need for a technique that facilitates chromosome spreads of plant cells on similar lines of mammalian chromosomes.

In spite of a number of investigations on the mechanism of banding in mammalian chromosomes<sup>44-50</sup>, what causes the banding is still obscure. While numerous such investigations are still being pursued, the lack of our understanding of the phenomenon of banding at present has not deterred the use of these techniques in chromosome research.

However, a thorough knowledge of the causes of banding production and factors influencing it may not only help in fostering the research on chromosome identification but also elucidate the structure and molecular organization of the chromosomes.

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# EFFECT OF IRRADIATION ON TRANSDUCTION AND LYSOGENISATION IN *SALMONELLA TYPHIMURIUM*

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## ABSTRACT

Irradiation of either temperate Phage 547 of *Salmonella* or recipient cells increases the frequency of transduction whereas lysogenization decreases. The increase in transduction is owing to the stimulating effect of the irradiation on recombination and loss of lytic action of the phage. The irradiation results suggest that the whole phage genome is more radio-sensitive than the transducing fragment.

## INTRODUCTION

IN the previous paper<sup>1</sup> it has been reported that wild type *S. typhimurium* strain 547 is lysogenic and releases temperate phage called P 547. This phage belongs to the class of general transducing temperate phages of *S. typhimurium* as it can transduce any marker. Transduction and lysogenization are shown to be independent processes in this case, unlike in lambda phage-*E. coli* system.

Zinder<sup>2</sup> studied the effect of irradiated phage P 22 of *Salmonella* on transduction and found a rise in the frequency as compared to that of un-irradiated phage. Similarly, an enhancement of sexual recombination was found by irradiation of the F<sup>+</sup> donor parent cells<sup>3</sup>. I wish to report in this paper the results of the effect of irradiated 547 phage and irradiated recipient cells on the efficiency of transduction and lysogenisation. It was hoped that this study might give some information on the nature of transducing fragment and phage genome.

## MATERIALS AND METHODS

The bacterial cultures, phage stock, media, and the techniques for transduction and lysogenization are described in the earlier paper<sup>1</sup>.

## RESULTS

**Effect of gamma irradiation on phage survival and adsorption.**—A suspension of 547 wild type phage in BHI (Brain heart Infusion broth) was irradiated at 200 kr, 400 kr and 600 kr in plastic tubes attached onto the window of Cobalt-60 radiation source. The ability of plaque formation on sensitive *S. typhimurium* (TC) cells and adsorption (on heat killed *S. typhimurium* 533 cells) was measured in control and irradiated phage samples. The effect of various doses of irradiation on per cent survival and adsorption are shown in Table I. The data showed that although

TABLE I

*Effect of gamma irradiation of 547 phage on survival, adsorption and its efficiency in transduction and lysogenisation*

Irradiation dose in kr.	% Survival <sup>a</sup>	% Adsorption <sup>b</sup>	H <sup>+</sup> -T <sup>+</sup> -L <sup>+</sup> transduction <sup>c</sup>	% Lysogeny <sup>d</sup>
0 (Control)	100.0	100	196.0	90.0
200	38.2	100	259.2	82.0
400	8.8	100	230.5	68.1
600	0.8	100	138.4	46.5

(a) Phage suspension with a titre of  $6.8 \times 10^8$ /ml was irradiated and titrated on T.C. cells.

(b) The percentage of adsorption of phage was determined on heat killed cells<sup>5</sup>.

(c) The multiplicity of infection (m.o.i.) was kept 0.3 and the number of transductions are expressed per  $10^8$  recipient cells.

(d) The percentage of lysogenisation was determined among the transduced cells.

there was a linear decrease in per cent survival of phage, when phage was irradiated at various doses, the adsorption remained to be 100% at these doses indicating that irradiation inactivated the phage genome which did not affect on the adsorption capacity.

**Effect of irradiated phage on transduction.**—The above irradiated samples were used for transduction experiments to see the correlation between the phage survival or inactivation, adsorption and ability to transduce by the irradiated phage. The frequency of general transduction (for His<sup>+</sup>, Try<sup>+</sup>, and Leu<sup>+</sup> markers) by control and irradiated 547 phage is shown in Table I. It is quite evident that irradiation of phage with 200 kr and 400 kr enhances the frequency of transductions as compared to un-irradiated phage. Garen and Zinder<sup>4</sup> found a similar effect of U.V. irradiated phage P 22 on transduction of these markers. The transduction frequencies have decreased below control level when the phage was irradiated at 600 kr dose (Table I). These results demonstrate that

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inactivated phage which has not lost the capacity of adsorption could transduce efficiently, *i.e.*, an active phage genome is not necessary for adsorption and transduction processes. Since the adsorption of irradiated phage is not increased, it is probable that irradiation itself makes the transduction process more efficient. To test this view, the transduction experiments were performed in which the U.V. irradiated recipient cells and unirradiated phage were employed.

*Effect of U.V. irradiation on survival of recipient cells and their ability to adsorb phage.*—First the rate of inactivation of recipient cells by U.V. irradiation and their capacity to adsorb phage were determined, the results of which are shown in Table II. After 60 and 90 seconds of irradiation,

TABLE II

*Comparative data on the effect of U.V. irradiation on survival of recipient cells, and their ability in adsorption and transduction*

U.V. dose in seconds	% Survival	% Adsorption	Transductions per 10 <sup>8</sup> cells
0 (Control)	100.0	100.0	517.3
60	26.2	100.0	886.0
90	13.7	100.0	593.0

Exponentially growing culture with a titer of  $2.4 \times 10^9$ /ml was suspended in chilled saline and was irradiated at a distance of 53 cm, with a 15 watt General Electric Germicidal lamp for above indicated times. All operations were carried out under red light to avoid photoreactivation. The above cultures were used for transduction experiments in which the m.o.i. was 2.3 in all the cases.

the cells were titrated for survival and the same cells were used for phage adsorption measurements. A control culture was run in parallel for comparison. The results in Table II show that 26.2% and 13.7% of cells survived when they were irradiated for 60 and 90 seconds. However, these cultures containing the mixture of active and inactive cells possessed the same capacity of phage adsorption as control. These results are analogous to those obtained by using heat killed cells which could still adsorb the phage without supporting replication<sup>5,6</sup>.

*Effect of U.V. irradiated recipient cells on transduction.*—The transduction experiments were carried out by using the above irradiated recipient cells and non-irradiated 547 phage. The data in Table II indicate that the transduction frequency has significantly increased (as it did for irradiated phage) when the indicator cells were irradiated for 60 and 90 seconds. The frequency of transduction

was lower in the case of 90 seconds irradiation than for 60 seconds, although both these values were much higher than the control. The reason for this decrease was that survival of recipient cells was lower at 90 seconds than at 60 seconds of irradiation (Table II). Thus the above results (in Tables I and II) indicated that irradiation of either phage or recipient cells enhanced the process of transduction without an increase in phage adsorption.

*Effect of irradiation on lytic action of phage and its effect on transduction.*—It is well established that in the temperate phage lysate only a small fraction of phage particles will constitute transducing phages and other fraction of the phages will either lysogenise or lyse the infected cells<sup>7</sup>. Watson<sup>6</sup> has shown that X-irradiation of T<sub>2</sub> phage concurrently diminishes lytic action and phage survival. The decrease in lytic action of irradiated temperate phage may be one of the factors in increasing transduction frequency in the preceding experiments (Table I). If the lytic action of phage is decreased by irradiation one can expect a higher percentage of survival of irradiated phage-infected cells than unirradiated phage-infected cells. To test this possibility the following experiment was performed to determine the lytic or killing action of control and irradiated phage on recipient cells by measuring the cell survival before and after infection.

The cells were allowed to infect with 200 kr, 400 kr and 600 kr irradiated and unirradiated phage samples and after 20 minutes of infection the unadsorbed phage was removed by centrifugation. The cell assays were made before and after infection of phage. The results in Table III show that

TABLE III

*Comparative data on the per cent survival of irradiated phage and cell survival after infection*

Irradiation dose in kr	% Phage survival	% Survival of infected cells	% Lysis of infected cells
0 (Control)	100.0	68.6	31.4
200	38.2	74.0	26.0
400	8.8	84.6	15.4
600	0.8	100.0	0.0

The m.o.i. was 0.36 in all four cases.

the per cent survival of infected cells increased as the function of irradiation dose, that is the decrease in cell lysis after infection with irradiated phage approximately paralleled the decrease in phage

survival after irradiation. From these results it appeared that the gamma-irradiation affected the lytic or killing property and the viability of 547 phage to the same degree. Contrary to this the host killing property of the virulent phage  $T_2$  is enormously greater in its resistance to U.V. irradiation than the viability of the phage<sup>8</sup>. This suggests that lysis of the cells by temperate phage may require phage reproduction but in the case of virulent phage nonviable phages may also kill or lyse the cells. Due to loss of host killing property of irradiated 547 phage there will be a greater chance for the phage to transduce a cell as compared to unirradiated phage, as a result, the frequency of transduction is increased in the former case.

*Effect of irradiated phage on lysogenisation.*—The fact that the frequency of transduction is not affected by the irradiated inactive phage, suggests that the genetic material of the phage alone and not the transducing chromosome fragment is inactivated by irradiation particularly at lower doses. If this is so, lysogenisation should decrease in the same order as phage inactivation since lysogenisation is established by the incorporation of the phage genome into bacterial chromosome<sup>9</sup>. In view of this, the effect of irradiated phage on lysogenisation frequency was determined (Table I). Unlike transduction, the frequency of lysogenisation steadily decreased as the irradiation dose increased. These results thus help in distinguishing the phage genetic material from that of the transducing fragment. The transduction and lysogenisation results reveal that transducing fragment is much less sensitive to irradiation than phage genome.

#### DISCUSSION

Why does gamma or U.V. irradiation of either phage or recipient cells enhance the transduction frequency though there is no increase in adsorption? One explanation suggested by Jacob, Wollman and Hayes<sup>3</sup> is that irradiation has a stimulating effect on the genetic recombination. In *Drosophila* high temperature and irradiation have been

shown to stimulate crossing over<sup>10</sup>. The data presented here support the notion that loss of lytic action or host-killing property of the irradiated inactive phage (but transducible) appears to be another important factor in increasing the transduction frequency by way of decreasing the cell lysis of the infected cells.

The data presented in the previous paper<sup>1</sup> that  $H^+-T^+-L^+$  markers were not carried on a single large transducing fragment, but instead each marker was present on a separate fragment. Some further evidence on the nature of the transducing fragment and phage genome may be deduced from the differences on the effect of irradiation on phage inactivation and its ability to transduce and lysogenize. Both phage survival and the lysogenisation decreased as the irradiation dose increased without a corresponding decrease in transduction. According to target theory<sup>11</sup> the smaller the action volume, the more resistant is the particle to irradiation, therefore, it may be suggested that the transducing DNA fragment is smaller than the genome of 547 phage.

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## RADIOECOLOGY OF SOME BLUEGREEN ALGAE

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### ABSTRACT

The bluegreen algae *Anacystis nidulans* and *Anabaena flos-aquae* absorb thorium by a passive process when the chemical is present in the medium. However, the two species differ significantly from each other in their capacity to absorb and accumulate the radiochemical, the effect of light on absorption, the highest concentration factor attained, etc., thereby showing that it is not safe to make generalised predictions about the reactions of any one radiochemical with regard to even closely related species of organisms.

### INTRODUCTION

SINCE algae are among the most important primary producers in the hydrosphere, their uptake of radioactive elements is of considerable importance to man, as has been stressed by many<sup>1-3</sup>. Algae sometimes accumulate radioactive substances in their cells to concentrations several hundred times more than that present in the surrounding water<sup>4-6</sup>, apparently for no special metabolic function<sup>7</sup>. By entering the food chains, these may cause serious hazards to man<sup>1,8-9</sup>. During an investigation into the effect of ionizing radiation of thorium on algae, it has been found that two species of the same group differ significantly in their capacity to absorb, accumulate and release the element. Thorium is of special interest because it is important in the nuclear energy field, as it can be transformed into fissile <sup>233</sup>U, and also because it is found in vast naturally-occurring radioactive deposits in several places on the Earth<sup>10</sup>.

### MATERIALS AND METHODS

Sterile cultures of the bluegreen algae *Anacystis nidulans* and *Anabaena flos-aquae* were grown in a slightly modified medium of Kratz and Myers<sup>11-12</sup> and in Allen and Arnon's medium<sup>13</sup> respectively. The cultures were prepared in such a manner to have, at the time of commencement of the experiment, 10 mg wet weight of algal cells per flask with 25 ml culture solution and were maintained at 15°-18° C under continuous fluorescent "white" light of 4,800 lux. Thorium was supplied to them in the form of thorium chloride aqueous solution with thorium concentrations of 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 5.0 mg, 7.5 mg, 10.0 mg, 15.0 mg, 20.0 mg and 25.0 mg per flask. Three such sets were prepared besides the control. After 2 hours they were washed and filtered through Millipore filter No. HAWP. 047-00, put into 10.0 ml of scintillating liquid and the counts determined using an ICN Tracerlab Scintillation Counter. Corrections for the slight quenching caused by the algal cells were determined with the help of a previously prepared graph. The experiments were per-

formed thrice with *A. nidulans* and *A. flos-aquae* cultures separately, using three replicates for every concentration of thorium. From the readings obtained the amount and percentage of thorium absorbed and the concentration factor were calculated.

### RESULTS AND DISCUSSION

The results from the experiment represented in Fig. 1 shows that the amount of thorium absorbed by *A. flos-aquae* is limited to about 1.7 mg thorium/10 mg wet weight of alga, irrespective of

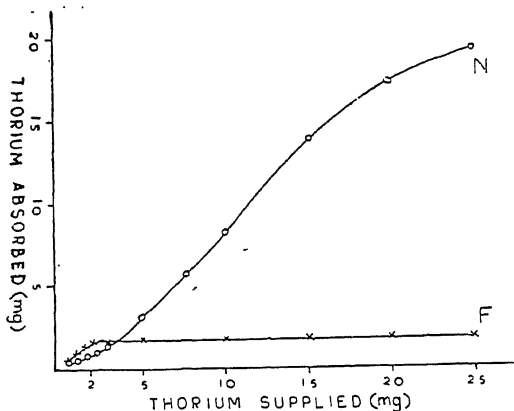


FIG. 1. The relation between concentration of thorium in the medium and the amount of thorium absorbed. (F = *Anabaena flos-aquae*; N = *Anacystis nidulans*.)

the increase in concentration of the chemical in the medium, while the capacity of *A. nidulans* to absorb thorium increased correspondingly with its concentration in the medium. The maximum amount of thorium absorbed by *A. nidulans* is found to be about 19.6 mg/10 mg wet weight of alga.

With an increase in the concentration of thorium in the medium the concentration factor decreases in both the species. Moreover, while the rate of this decrease is slow in *A. nidulans* it is quite rapid in *A. flos-aquae* as shown in Fig. 2.

Although greater amounts of thorium are absorbed by *A. nidulans* at higher concentrations, the highest

concentration factor of 8,248 was recorded in *A. flos-aquae* in comparison with the highest value of 6,540 in *A. nidulans*.

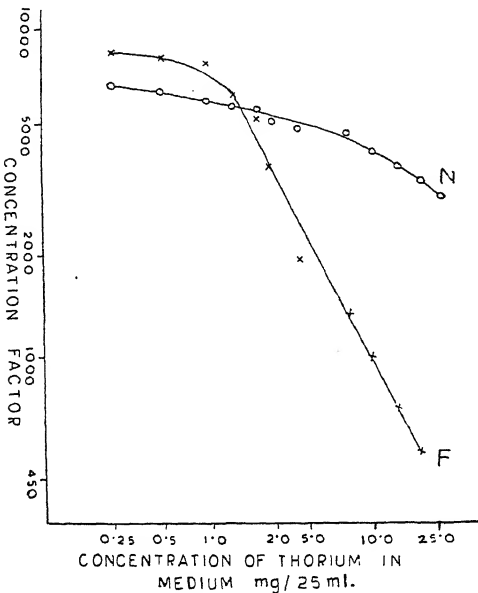


FIG. 2. The relation between concentration of thorium in the medium and the concentration factor. (F = *Anabaena flos-aquae* ; N = *Anacystis nidulans*.)

The absorption of thorium by both the species of bluegreen algae is by a passive process. The maximum concentration factor is attained in both species within 70 minutes of treatment with thorium. Contrary to expectation it has been found that in comparison with absorption, the quantity of the chemical adsorbed by the cells is negligible, being always below an average of 1.9%.

The sorption of some radiochemicals by dead cells, dividing cells and nondividing cells have been studied comparatively by some earlier investigators<sup>14-15</sup>. The pH of the medium is found to affect the absorption and retention of thorium by *A. flos-aquae* and *A. nidulans*. When the medium is made acidic with three drops of conc. H<sub>2</sub>SO<sub>4</sub> per 25 ml of culture solution containing 10 mg wet weight of algae, 95%–97% of the absorbed thorium is released back into the medium by the cells within 5 minutes.

The uptake of radiochemicals such as strontium by marine algae is reported to be affected by light<sup>16</sup>. It is a common practice<sup>1</sup> that in terms of relation to light the radioactive substances are divided into two categories: (1) concentration depending on light and (2) concentration not depending on light. The results of an experiment with *A. nidulans* and *A. flos-aquae* grown in complete darkness and

normal light, presented in Table I, reveals that light increases the rate of absorption of thorium to a small extent with *A. nidulans* but has no effect in the case of *A. flos-aquae*. This shows that the concentration of the radiochemical by the organism depends more upon the species concerned than on the presence or absence of light.

TABLE I  
Comparison of concentration factor in cells grown in light and in darkness

Name of species	Concentration factor in cells grown in light	Concentration factor in cells grown in darkness
<i>A. nidulans</i> ..	4,902	4,430
<i>A. flos-aquae</i> ..	1,943	1,953

From the above experiments it becomes evident that it is not safe to make generalised predictions about the reactions of any one radiochemical with regard to even closely related species of organisms. This fact is important in the present context of increasing radiation pollution of the hydrosphere resulting from the production of radioactive wastes by the nuclear power industry and the radioactive fallout following nuclear weapon tests.

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# ON THE POINT OF ORIGIN OF HEART BEAT IN THE SCORPION, *HETEROMETRUS FULVIPES* C. KOCH

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## INTRODUCTION

IN scorpions the heart beat is known to be neurogenic as evidenced by the presence of a cardiac ganglion and its spontaneous rhythmic burst activity related to the contractions of the heart muscle in 1:1 ratio<sup>1,2</sup>. Since the heart of the scorpion is tubular and the cardiac ganglion is on the mid-dorsal line of the heart, it is of interest to find whether the generation of the spontaneous impulses from the cardiac ganglion is simultaneous throughout the ganglion or spread from one region to the other in a regular sequence.

Previous work on scorpions showed that the heart beat is maximum in the posterior bits of the heart compared to those in the anterior bits<sup>3,4</sup>. Similarly alterations in the activity of the cardiac ganglion in the anterior and posterior parts have also been reported<sup>3</sup>. The present study is aimed at finding of such gradient activities in the heart beat and the cardiac ganglion in the scorpion are related.

## MATERIAL AND METHODS

Hearts were isolated from the scorpions belonging to *Heterometrus fulvipes* C. Koch and maintained in the ringer medium<sup>5</sup>. Spontaneous electrical activity of the cardiac ganglion was studied by recording the electrical activity at different regions. The differences in characteristics like burst frequency, burst amplitude, burst duration and interburst intervals in the spontaneous electrical activity of the cardiac ganglion recorded at various levels were determined by analysing the recorded film.

Acetylcholinesterase (AChE) activity was determined in the two halves of the heart muscle based

on the method of Metcalf<sup>6</sup>. Succinic dehydrogenase (SDH) activity was estimated by the application of the method described by Nachlas *et al.*<sup>7</sup>. Protein content of the heart muscle was estimated by the method of Lowry *et al.*<sup>8</sup> to represent the enzyme activities per milligram protein.

## RESULTS AND DISCUSSION

Earlier observations by the author<sup>9</sup> on the heart beat of the scorpion, *H. fulvipes*, indicated that there is a regular sequence in the cardiac cycle. The peristaltic wave commenced at the posterior part of the heart, and it gradually spread to the anterior end of the heart. The extent of contraction and relaxation of the heart decreased in the postero-anterior direction of the heart.

Recordings of the spontaneous electrical activity at different regions of the cardiac ganglion showed the presence of a postero-anterior gradient as in Fig. 1. The activity was maximum at the 7th ostial region of the cardiac ganglion and it gradually decreased anteriorly. As such, the activity was mostly in the form of regular bursts with few units firing in the interburst period at the posterior part of the ganglion. Such a typical burst of activity disappeared gradually towards the anterior end, especially at the 1st ostial region of the ganglion.

The characteristics of the burst also showed variation at different regions of the cardiac ganglion (Fig. 2). Burst frequency did not show any typical gradient and it was more or less fluctuating between 60-68 bursts/min (Fig. 2a). The burst amplitude, however, showed a typical trend in that it decreased in the postero-anterior direction. It had a maximum burst amplitude value of 580  $\mu$ Volts at the 7th ostial region whereas the burst

amplitude at the 1st ostial region was only 150  $\mu$  Volts (Fig. 2 b). Besides, the number of units firing in a burst also decreased postero-anteriorly.

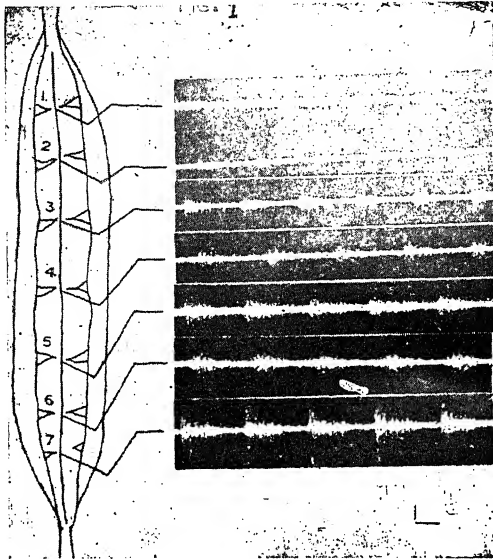


FIG. 1. Spontaneous electrical activity recorded from different regions (as indicated in the figure given on the left side) of the cardiac ganglion to show the postero-anterior gradient. Time: 0.5 sec; Calib: 50  $\mu$ V.

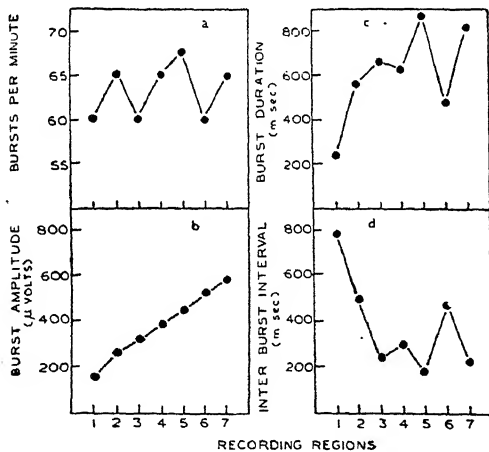


FIG. 2. Graph showing the differences in the various parameters of the spontaneous activity recorded at different regions of the cardiac ganglion.

The units firing in the interburst interval also decreased both in number and amplitude towards the anterior end of the cardiac ganglion. The burst duration was also fluctuating around a mean value of 670 m. sec. at all regions excepting at the 1st ostial region where the burst duration decreased to 270 m. sec. (Fig. 2 c). The interburst interval

was low at the posterior region of the cardiac ganglion. The average interburst interval was 285 m. sec. from the posterior region up to the 3rd ostial region. However, the interburst interval increased at the anterior region of the cardiac ganglion where it was 500 m. sec. and 800 m. sec. at the 2nd and 1st ostial regions respectively (Fig. 2 d).

AChE enzyme activity in the heart was maximum in the posterior half of the heart, and less so in the anterior half (Table I). The differences in the enzyme activities in the two halves of the heart were statistically checked and found to be significant.

TABLE I

Acetylcholinesterase and succinate dehydrogenase activities in the anterior and posterior halves of the heart values expressed are the average  $\pm$  S.D. of 6 observations

	Acetylcholinesterase*		Succinate dehydrogenase†	
	Anterior half of the heart	Posterior half of the heart	Anterior half of the heart	Posterior half of the heart
Enzyme activity	155.71	214.273	3.488	5.922
't' value	$\pm 8.65$	$\pm 10.35$	$\pm 0.24$	$\pm 0.49$
	$t = 9.6954$		$t = 9.9633$	
	$p < 0.001$		$p < 0.001$	
Inference	Activity more in posterior part		Activity more in posterior part	

\* Acetylcholinesterase =  $\mu$  moles of Ach hydrolysed/mg protein/hr, † Succinate dehydrogenase =  $\mu$  moles Formazon/mg protein/hr.

It is of interest to note that electrical activity of the cardiac ganglion, enzyme activities of the heart muscle and the heart beat follow similar activity patterns. A typical postero-anterior gradient is seen. An inference is that the activity levels may be related to one another and perhaps inter-dependent.

It is suggested that the rhythmicity in various physiological processes of the scorpion might be due to rhythmicity in the central nervous system<sup>10</sup>. Such rhythmicity in the central nervous system may be brought about by the agency of neurosecretory material of the cephalothoracic nerve mass<sup>11</sup>. The effect of neurosecretory material on the AChE activity of the central nervous system and the enzyme present in the central nervous system of the scorpion are said to be closely related to the electrical activity of the central nervous system<sup>12-13</sup>. As in the above the rhythmicity of the heart beat which has been shown to be influenced by the rhythmicity of the cardiac ganglion, may be influenced by a neurosecretory system.

It is known that AChE is found in the nervous tissues of invertebrates<sup>14</sup>. It is also known that

the levels of activity of the nervous system is related to the activity of the enzyme<sup>10</sup>. The enzyme activity is related to the amount of Ach synthesized and released. Since the electrical activity of the cardiac ganglion shows variations it is reasonable to expect changes in the AChE activity also. High level of activity necessitates the availability of energy in larger amounts which steps up the oxidative pathways. Besides, high levels of Ach, leading to increased AChE activity, it also stimulates the cellular respiration leading to release of higher amounts of energy<sup>15</sup>. Thus the variations in the heart beat at different regions seem to be due to the acetylcholine synthetic and energy yielding processes leading to variations in the electrical activity of the cardiac ganglion.

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is dedicated to the sweet memory of late Prof. K. Pampapathi Rao.

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### CHEMICAL COMPONENTS OF *EUGENIA JAMBOLANA* STEM BARK

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*EUGENIA JAMBOLANA* (Syn. *Syzygium cumini*) belongs to the family Myrtaceae and the common Indian name is 'Jamun'. The stem bark was examined by Sengupta and Das<sup>1</sup> who isolated betulinic acid, friedelin, epi-friedelanol,  $\beta$ -sitosterol and eugenin, a fatty acid ester of epi-friedelanol. However the name eugenin for the ester was not appropriate since it had already been used long ago for 2-methyl-5-hydroxy-7-methoxy chromone which was isolated from *Eugenia caryophyllata*<sup>2,3</sup>. The bark is astringent and is used in sore throat, bronchitis, asthma and dysentery; therefore in order to study its phenolic constituents, the stem bark (400 gm) was cut into coarse powder and extracted with petroleum ether and alcohol. Identification of compounds was confirmed whenever possible by direct comparison with authentic samples using mixed m.p. and I.R. spectra.

The petroleum ether extract yielded a brown oil (15 g) which was subjected to column chromatography over silica gel using petroleum ether-benzene as eluants and 4 compounds A, B, C, D were obtained. Compound A (500 mg), crystallised from chloroform-methanol as colourless needles, m.p. 309–

311°,  $[\alpha]_D + 7^\circ$  ( $\text{CHCl}_3$ ) and was identified as betulinic acid. Compound B, after crystallisation from  $\text{CHCl}_3$ -MeOH (350 mg) as needles had m.p. 255–56°  $[\alpha]_D - 20^\circ$  ( $\text{CHCl}_3$ ). It gave positive Liebermann-Burchard test. It was identified as friedelin. Compound C (50 mg) crystallised from  $\text{CHCl}_3$ -MeOH as needles, m.p. 295–300°,  $[\alpha]_D + 14^\circ$  ( $\text{CHCl}_3$ ). Its identity as friedelan-3  $\alpha$ -ol was confirmed by comparison with an authentic sample, and by the preparation of its acetate. Compound D, crystallised from methanol as colourless needles (70 mg), m.p. 135–36°,  $[\alpha]_D - 30.6^\circ$  and it was identified as  $\beta$ -sitosterol.

*Alcohol extract.*—From the alcohol extract, was obtained ethyl acetate soluble and ethyl acetate insoluble fractions. The former was concentrated to a syrup which was a mixture of three compounds (TLC). They were separated by column chromatography over silica gel using ethylacetate and different proportions of ethyl alcohol as eluates to yield E, F and G. Compound E (200 mg), crystallised from aqueous ethanol and had m.p. 177–179°. It gave a brown colour with alcoholic ferric chloride and deep red colour with magnesium and



hydrochloric acid,  $\lambda_{\text{max}}^{\text{MeOH}}$ : 264, 367 nm. It formed an acetate by the pyridine-acetic anhydride method and crystallised from ethyl acetate as needles, m.p. 186–187°. The compound was identified as kaempferol by comparison of itself and of its acetate with authentic kaempferol and its tetra-acetate respectively. Compound F crystallised from alcohol, m.p. 315–317°,  $\lambda_{\text{max}}^{\text{MeOH}}$ : 255, 302, 317 nm. It formed an acetate with pyridine/acetic anhydride, m.p. 199–200°. The compound F was identified as quercetin. Compound G (70 mg), crystallised from pyridine-alcohol. It gave positive LB test. when hydrolysed with 7% aq. methanolic (50/50 v/v) sulphuric acid, it gave  $\beta$ -sitosterol as the aglycone and D-glucose as the sugar part. It gave an acetate, m.p. 166–167°,  $[\alpha]_D -40^\circ$  ( $\text{CHCl}_3$ ). The parent compound and its acetate were identical in all respects with authentic samples of  $\beta$ -sitosterol- $\beta$ -D-glucoside and its acetate respectively.

The ethyl acetate insoluble fractions yielded two compounds H and I separated by preparative chromatography (TLC). Compound H (20 mg) crystallised from ethanol as pale yellow prisms, m.p. 176–179°. It gave brownish green ferric chloride reaction and deep red colour with magnesium-hydrochloric acid. On acid hydrolysis it gave kaempferol as the aglycone. The aq. solution contained D-glucose. The glycoside gave the

following UV data:  $\lambda_{\text{max}}^{\text{MeOH}}$  265, 350 nm, + NaOAc 272, 302, 375 nm, + NaOAc +  $\text{H}_3\text{BO}_3$  265, 302, 375 nm, +  $\text{AlCl}_3$  273, 302, 393 nm. It was identical with kaempferol-3-O-glucoside. The compound I (15 mg) was identified as sucrose.

A small portion of alcoholic extract was directly hydrolysed with 10% sulphuric acid and the hydroxylate was extracted with ethyl acetate. The ethyl acetate solution was found to be mixture of  $\beta$ -sitosterol, kaempferol, quercetin, gallic acid and ellagic acid. The last two showed the presence of gallo- and ellagi-tannins in the alcohol extract as major components. Boiling the alcohol extract with HCl did not produce any appreciable red colour indicating absence of proanthocyanidin.

Thus the present investigation on the stem bark of *E. jambolana* has revealed the presence of  $\beta$ -sitosterol-D-glucoside, kaempferol-3-O-glucoside, kaempferol and quercetin which were not noted before. Gallo- and ellagi-tannins which are present in the alcohol extract may be responsible for the astringent property of the stem bark.

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## LETTERS TO THE EDITOR

### VISUAL METEORIC ACTIVITY OVER WALTAIR DURING OCTOBER-FEBRUARY, 1961-1972

SRIRAMA RAO *et al.*<sup>1</sup> carried out systematic visual observations of meteors over the low latitude station Waltair (Lat. 17° 43' N) during November, December and January 1961-67 and presented monthly survey curves with the data of 7987 meteors. Similar observations during October and February 1961-72 yielded data of 1835 and 1108 meteors in the two months respectively. A representative and consolidated survey curve of visual meteoric activity over Waltair for the five months from October to February is now presented in Fig. 1. The method of Srirama Rao *et al.*<sup>1</sup> has been followed for analysis of the data of visual meteors for October and February. The daily mean hourly rates of visual meteoric activity from Olivier's<sup>2</sup> data from high latitudes ranging from 20° N-50° N for the years 1958-63 have also been presented for comparison.

There is good agreement in the general trends of the two curves in Fig. 1, though the rates, in general,

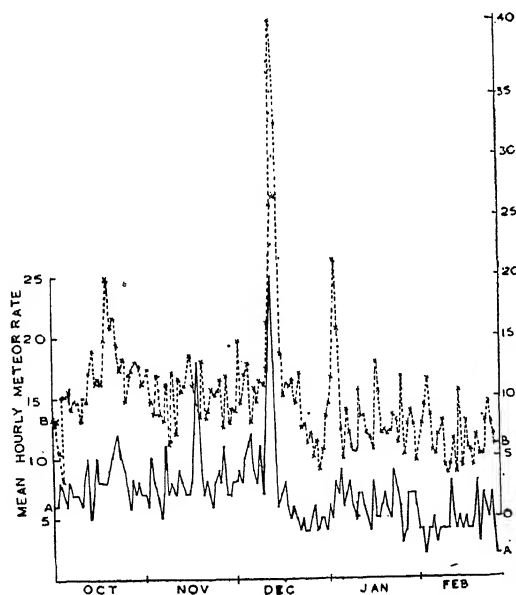


FIG. 1. Single observer visual meteor rates at Waltair (Curve AA) and at higher latitude stations (Curve BB) for the five months October to February.

are higher at higher latitudes as seen from Table I which summarises the monthly mean hourly rates

TABLE I  
Average monthly mean hourly meteor rates

Month	Waltair	Higher latitudes
October	8	11
November	8	10
December	9	13
January	6	9
February	5	7

for the two cases. It may be seen from Table I that the activity is, in general, higher during the last three months of the year at any place as compared with the first two months of the year. This trend in meteoric activity is also observed by earlier workers like Wolf, Olivier, Denning, and Schmidt (Lovell, A. C. B., 1954, p. 96).

The meteoric activity in October, November and December is augmented by the presence of the well-known major meteor showers, namely, the Orionids (peak activity on 22nd October), the Leonids (of 17th November) and the Geminids (of 12th December). The Taurids are also known to be active from about 15th October and throughout November. The Quadrantids (of 3rd January) are prominent at higher latitudes owing to the high declination of their radiant. No major meteor shower is observed in February. Some minor showers have also been detected during these months by Srirama Rao *et al.*<sup>3,4</sup>.

Space Res. Lab., M. SRIRAMA RAO.  
Andhra University, A. GOPALAKRISHNA MURTHY.  
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May 21, 1974.

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### NEW COMPOUNDS OF THE TYPE $A^+A^{3+}B^{4+}O_4^{2-}$ I STRUCTURE

LARGE number of compounds with the general formula  $AA'BO_4$  where  $A = Ln^{3+}$ ,  $A' =$  a vacance,  $B = W$  (1);  $A = Zn$ ,  $A' = Li^+$ ,  $B = V$ ,  $Nb$  (2) and  $A = Ln^{3+}$ ,  $A' = Li$ ,  $B = Si$ ,  $Ge$  (3) have been described in the literature<sup>1-3</sup>. These crystallise either

with the Scheelite ( $\text{CaWO}_4$ ) type<sup>4</sup>, fergusonite type<sup>5</sup> ( $\text{YTao}_4$ ) or phenacite type<sup>2</sup> of structures. However, compounds having the general formula  $\text{A}^+\text{A}^{3+}\text{B}^{4+}\text{O}_4^{2-}$ , where  $\text{A}^+ = \text{Li}, \text{Na}, \text{K}$ ,  $\text{A}^{3+} = \text{Ln}^{3+}$ , i.e., a rare earth cation and  $\text{Y}^{3+}$  and  $\text{B}^{4+} = \text{Ti}, \text{Hf}$ , and  $\text{Zr}$ , have not yet been reported. This note reports on the preparation and structural characteristics of compounds of the above type.

The pure components  $\text{Ln}_2\text{O}_3$  (fluka grade),  $\text{A}_2\text{O}$  or  $\text{A}_2\text{CO}_3$  (A.R. grade) and  $\text{BO}_2$  (A.R. grade) were weighed in the correct stoichiometric proportions, thoroughly mixed under acetone in an agate mortar and pestle and fired in platinum crucibles at 900–1300°C in high temperature furnace. Lattice parameters were determined by powder diffraction patterns of the products on a 14 cm diameter Debye-Scherrer camera using Ni filtered  $\text{CuK}_\alpha$  radiation. The lattice parameters are accurate to within  $\pm 0.01 \text{ \AA}$ .

Table I shows the lattice parameters and structure type for a few of the representative samples in this series. It can be seen that not all the compounds crystallise with the scheelite ( $\text{CaWO}_4$ )

TABLE I  
Lattice parameters and structure type of  
 $\text{A}^+\text{A}^{3+}\text{B}^{4+}\text{O}_4^{2-}$  compounds

Compound	Structure type*	Unit cell parameters $\text{\AA}$		$r_A/r_B$
		$a_T$	$c_T$	
$\text{LaNaTiO}_4$	.. F	monoclinic		1.544
$\text{NdLiTiO}_4$	.. F	monoclinic		1.640
$\text{NdLiHfO}_4$	.. S	5.13	11.08	1.376
$\text{SmNaTiO}_4$	.. S	5.12	11.24	1.463
$\text{SmKHfO}_4$	.. S	5.16	11.35	1.463
$\text{GdNaTiO}_4$	.. S	5.11	11.12	1.448
$\text{GdKZrO}_4$	.. S	5.16	11.37	1.469
$\text{DyNaTiO}_4$	.. S	5.14	11.06	1.426
$\text{DyKZrO}_4$	.. S	5.16	11.38	1.450
$\text{YNaTiO}_4$	.. S	5.13	11.04	1.382
$\text{YKTiO}_4$	.. F	monoclinic		1.662

\* F=Fergusonite, S=Scheelite.

structure. It is also observed that this structure is stable within  $1.376 \leq r_A/r_B \leq 1.469$ , the value for  $\text{CaWO}_4$  taking Ahrens<sup>6</sup> radii into consideration being 1.456. The  $\text{YTao}_4$  lattice is stable within  $1.544 \leq r_A/r_B \leq 1.662$  as compared to 1.613 found<sup>5</sup> for fergusonite structure. The 'a' and 'c' parameters of the scheelite lattice exhibit a trend reminiscent of the lanthanide contraction in that plots of  $a_T$  and  $c_T$  as a function of the atomic number of the rare earth ions, keeping the B ions fixed, exhibit a slight curvature at Dy. These results are in agreement with our previous observations on the  $\text{Li}_{0.5}\text{Ln}_{0.5}\text{TiO}_3$  systems<sup>7,8</sup>. These composi-

tions exhibit interesting electrical and magnetic properties which are reported elsewhere<sup>9,10</sup>. The possibility of using these compositions as dielectric capacitors is also explored.

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#### PHYSICOCHEMICAL STUDIES ON N-ACETYL-PHENYLHYDROXYLAMINE METAL COMPLEXES

THE equilibrium studies of the metal complexes of the ligands containing  $-\text{CO}-\text{N}(\text{OH})-$  as the electron donor group have recently come more and more into the foreground of interest. Dutta and Seshadri<sup>1</sup> as well as Jaimini and Sogani<sup>2,3</sup> have collected physicochemical data on some metal chelates of a few compounds having the above functional group. Literature survey indicates that N-acetyl-phenylhydroxylamine (N-APHA), a ligand utilised in the spectrophotometric determination of iron<sup>4</sup> has not yet been used for the physicochemical study of its metal chelates. The object of this communication is to evaluate stability constants values of Cu(II), Ni(II), Co(II), Mn(II) and Zn(II) chelates of N-APHA by employing Calvin-Bjerrum<sup>5,6</sup> pH-titration technique.

*Experimental.*—All chemicals used were of analytical reagent grade. N-APHA was prepared by the earlier reported method<sup>4</sup>. Dioxane was purified by Weissberger's method<sup>7</sup>. Standard solutions of carbonate free sodium hydroxide, perchloric acid and metal perchlorates were used for measurements. Neutral sodium perchlorate solution was used to maintain constant ionic strength at 0.1 M. pH measurements were made using a cambridge bench

type pH-meter with glass and calomel electrode assembly. The pH-titrations were carried out in 50% v/v dioxane-water medium. Reaction solutions were all thermostated at  $25 \pm 0.1^\circ \text{C}$ .

The experimental procedure involved potentiometric titration of the ligand with standard alkali solution in the absence of, and in the presence of, the metal ions studied. These included titrations of (i)  $\text{HClO}_4$  ( $5.0 \times 10^{-4}$  moles) and reagent ( $2.0 \times 10^{-4}$  moles); and (ii)  $\text{HClO}_4$  ( $5.5 \times 10^{-4}$  moles), reagent ( $2.0 \times 10^{-4}$  moles) and metal ion ( $2.0 \times 10^{-5}$  moles) against 0.1 M sodium hydroxide solution. The initial volume of the solution was 100 ml. The pH-meter readings obtained after equilibrium at a given alkali addition in duplicate titrations were reproducible with a maximum variation of  $\pm 0.02$  pH unit. Necessary pH correction<sup>8</sup> was made for using 50% v/v dioxane-water mixture.

**Results and Discussion.**—The possibility of hydrolysis of these metal ions in presence of a large excess of the ligand is ruled out, as there was no possible indication in the trend of pH changes during titration. The plots of pH (corrected) of the solutions at each equilibrium point against the volume of the alkali added were drawn. The shapes of the titration curves were as usual. In any titration, 0.5 millimole of base were required to neutralise the excess perchloric acid and any protonated species which might be present; consequently, the displacement of the N-APHA, perchloric acid and metal ion curve at 0.5 millimole of base from the N-APHA and perchloric acid curve was due to proton release, which in turn depended on the reaction between the N-APHA and the metal ion. The presence of 0.02 millimole of metal ions required 0.04 millimole more of standard base to reach the neutralisation point. This implied that two equivalents of hydrogen ions had been released per mole of metal ion in solution. Thus, it was concluded that all complexes were 2 : 1, N-APHA to metal ion.

The dissociation constant,  $\text{p}K_a$  of N-APHA and  $\bar{n}$  -  $\text{pR}^-$  data pairs corresponding to the formation curves (Fig. 1) were calculated from the pH-metric titration data in the usual way<sup>6</sup>. The  $\log K_1$  and  $\log K_2$  for Cu (II), Ni (II), Co (II) and Zn (II) chelates were directly read from the formation curves. These values for Mn(II) chelate were calculated by the method of least squares<sup>9</sup>. The results are given in Table I.

The  $\bar{n}$  values for complexes of all systems show that they are greater than 1 and less than 2, indicating the formation of 1 : 1 and 1 : 2 complexes.  $\text{p}K_a$  value of N-APHA was potentiometrically found to be 9.76. From Table I, the order of stability

is  $\text{Cu} > \text{Ni} > \text{Co} > \text{Zn} > \text{Mn}$ , which agrees with the one given by Mellor and Maley<sup>10</sup>. An approximate parallelism between  $\log K_n$  and second ionisation potential as well as the usual correlation between the former and the atomic numbers were observed.

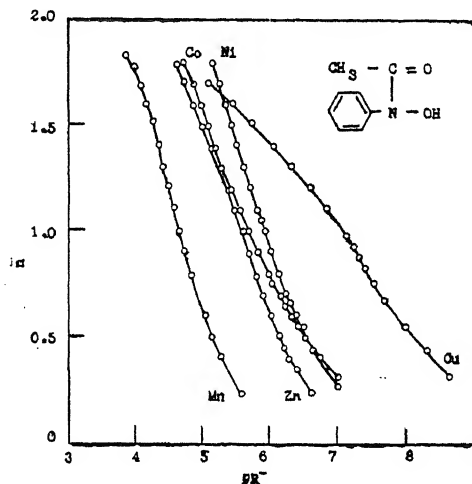


FIG. 1. Formation curves for binding N-APHA by metal ions.

TABLE I  
Stability of metal chelates of N-APHA

Metal	$\log K_1$	$\log K_2$	$\log \beta_2$
Cu	8.18	5.64	13.82
Ni	6.58	5.40	11.98
Co	6.46	5.30	11.46
Zn	6.18	4.94	11.12
Mn	5.07	4.42	9.49

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Jadavpur University,  
Calcutta-32, March 13, 1974.

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S. LAHIRI.

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### POLAROGRAPHIC STUDY OF THE ACID HYDROLYSIS OF KETOXIMES

IN the course of polarographic studies on substituted acetophenone oximes in moderately concentrated hydrochloric acid solutions, the authors noticed a decrease in limiting current ( $i_1$ ) of the reduction wave with time. This change in limiting current was confirmed polarographically to be due to the hydrolysis of the oxime into ketone and hydroxylamine. It was also observed that the rate of hydrolysis shows a maximum at a particular acid concentration, which varied with the nature and position of the substituent in the substrate. In view of this the authors have undertaken the investigation on the acid hydrolysis of substituted acetophenone oximes by the polarographic method. The results obtained are presented in this communication.

**Experimental.**—The oximes studied namely, acetophenone oxime (I), 2-OH acetophenone oxime (II), 2-OCH<sub>3</sub> acetophenone oxime (III), 2, 4-dihydroxy acetophenone oxime (IV) and 2-OH, 4-OCH<sub>3</sub> acetophenone oxime (V) were prepared by the usual methods and their purity checked by comparing the physical constants with the literature data. The hydrolysis was followed by recording the current-voltage curves at definite time intervals and the first order rate constants were computed from the log  $i_1$  vs  $t$  plots. A LP 55 A photographic recording polarograph has been used. The capillary characteristics are  $m = 2.06$  mg/sec and  $t = 3.7$  sec/drop. The limiting current is found proportional to the concentration of the oxime. The variation of rate constant with acid concentration in respect of 2, 4 dihydroxy acetophenone oxime is presented in Table I as a typical representative. The effect of

ionic strength and organic cosolvent (methanol) on the rate constant are also exemplified by the data obtained with 2, 4-dihydroxy acetophenone oxime in Tables II and III. Similar behaviour was shown by other oximes.

All the oximes investigated exhibited a rate maximum. This observation is similar to the behaviour shown by moderately basic substrates, e.g., amides<sup>1-3</sup>, esters<sup>4</sup>, etc., in acid hydrolysis. The position of the rate maximum is dependent on the nature and position of the substituent. The fact that the rate exhibited a maximum indicates that two different rate determining steps are operative in the two acid ranges. Similar behaviour was reported by C.J.O'Connor *et al.*<sup>5</sup>, in the acid hydrolysis of *N*-*t*-butyl benzaldoxime and Moodie *et al.*<sup>6</sup> in the case of *p*-substituted acetophenone oximes. The influence of temperature, ionic strength and solvent was studied at acid concentrations falling on either side of the maximum. The rate decreased with increase of organic co-solvent, methanol in both the

TABLE I

Rate constants for hydrolysis of 2, 4-dihydroxy acetophenone oxime in hydrochloric acid at 30° C

[HCl] M	$k \times 10^4$ (Sec <sup>-1</sup> )	[HCl] M	$k \times 10^4$ (Sec <sup>-1</sup> )
0.04	0.64	0.7	1.35
0.1	1.02	1.0	1.15
0.4	1.42	2.0	0.69
0.5	1.45	3.0	0.63

TABLE II

Effect of ionic strength

[HCl] = 0.1 M		Temp. = 30° C.		
[KCl] M	$k \times 10^4$ (Sec <sup>-1</sup> )	0	0.2	0.4
		1.02	1.04	1.13
				1.76

TABLE III

Effect of organic solvent (Methanol)

[HCl] = 0.1 M		Temp. = 30° C.			
% by volume of alcohol	$k \times 10^4$ (Sec <sup>-1</sup> )	0	10	30	50
		1.02	0.84	0.49	0.13

TABLE IV

Oxime	Acid conc. at rate max. [HCl] in M	Lower acid			Higher acid		
		$k \times 10^4$ (Sec <sup>-1</sup> )		$\Delta E$ (K cal. mol <sup>-1</sup> )	$k \times 10^4$ (Sec <sup>-1</sup> )		$\Delta E$ (K cal. mol <sup>-1</sup> )
		At 35° C	At 40° C		At 35° C	At 40° C	
APO	0.4	9.60	12.57	10.4	11.34	14.60	9.7
2-OH APO	1.0	4.21	5.81	12.4	3.56	5.24	14.8
2, 4 (OH) <sub>2</sub> APO	0.3	1.46	2.28	16.9	1.73	2.65	16.4
2-OCH <sub>3</sub> APO	0.5	3.33	4.07	7.7	2.24	3.19	13.6
2-OH, 4-OCH <sub>3</sub> APO	0.4	1.57	2.02	9.6	2.30	2.92	9.0

APO = Acetophenone oxime,

acid regions and there is a linear relation between  $\log k$  and mole fraction of water. This suggests that water acts as a nucleophile in both the ranges. Since acid catalysis is observed in the lower acid range, it can be assumed that the protonation of the oxime is an essential step. Hence the rate limiting step can be assumed to involve the protonated oxime and the water molecule. Since the activity of water decreases with increase of acid concentration, the decrease in rate with acid concentration in the higher acid range suggests that the general base (water) catalysis is operative in this range. The reaction in the higher acid range can, therefore, be assumed to be a base (water) catalysed loss of the hydroxylamine from the protonated tetrahedral intermediate.

In general it is observed that unsubstituted acetophenone oxime has the highest and 2, 4-dihydroxy acetophenone oxime, the lowest rate of hydrolysis at all acid concentrations studied.  $-\text{OH}$  and  $-\text{OCH}_3$  groups in the 4-position decreased the rate of  $2-\text{OH}$  acetophenone oxime. This shows that electron donating groups retard the reaction. Similar observation was also made by Moodie *et al.* (*loc cit.*). But a substituent in the ortho position considerably decreased the rate of hydrolysis of the acetophenone oxime. This may be attributed to the ortho effect such as chelation or forcing of the benzene nucleus out of the plane of the oxime group and thus sterically inhibit the attack by water molecule.

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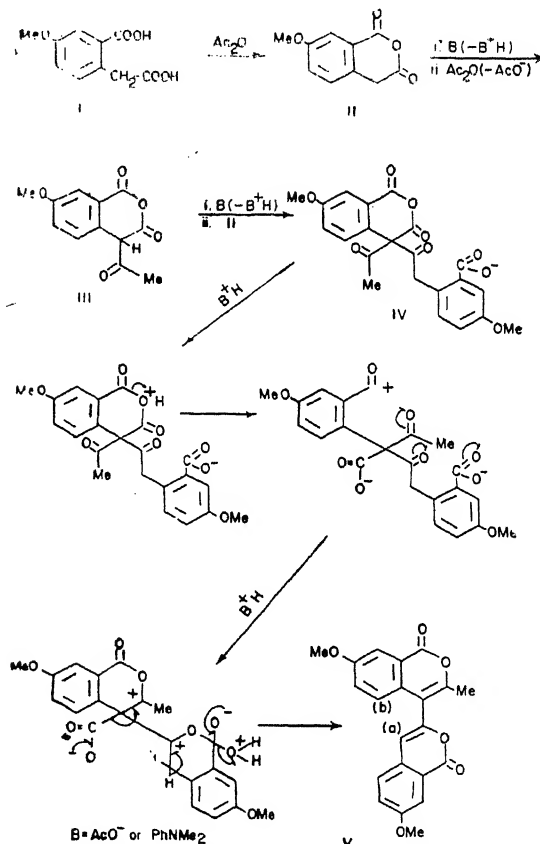
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#### ISOCOUMARINS: 4-(7-METHOXYISOCOUMARIN-3-YL)-7-METHOXY-3-METHYL-ISOCOUMARIN

In an earlier paper<sup>1</sup> we had reported the isolation of a small amount of a neutral substance A with m.p. 234–35 from the condensation of 4-methoxyhomophthalic acid (I) with acetic anhydride in presence of fused sodium acetate, in which 4-

carboxy-7-methoxy-3-methylisocoumarin was the major product. We recently observed that the same substance A is obtained in better yield from the condensation carried out in presence of N,N-dimethyl aniline (given below) in which also 4-carboxy-7-methoxy-3-methyl-isocoumarin was the other product with lot of tarry material.

The constitution of 4-(7-methoxyisocoumarin-3-yl)-7-methoxy-3-methylisocoumarin (V) is now assigned to the substance A and its formation is envisaged to have taken place as given below. The constitution was arrived at on the basis of elemental analysis, its I.R. and N.M.R. spectra.



The 4-acetylisochromandione III gets further acylated by the unchanged II and the resulting product IV, then undergoes rearrangement and lactonisation to give the isocoumarinylisocoumarin V. The reaction finds similarity to the earlier reported<sup>1</sup> formation of 7-methoxy-4-acetylisocoumarin as one of the products in the condensation of I with acetic anhydride in presence of pyridine at boiling water-bath temperature. In that case as has been explained<sup>2</sup> before, the initially formed III is

further acetylated at the reactive  $-\text{CH}-$  and then rearranges. The formation of V in the present reaction seems to have got promoted due to slow acetylation of II in presence of sodium acetate and N, N-dimethylaniline.

The substance A analysed constantly for  $\text{C}_{21}\text{H}_{16}\text{O}_6$  that is in conformity with V. Its IR spectrum showed bands at 1740 (lactonic  $>\text{C}=\text{O}$ ), 1718 (lactonic  $>\text{C}=\text{O}$ ) with 1618 ( $>\text{C}=\text{C}<$ ), 1560, 1510, 1490  $\text{cm}^{-1}$  (aromatic). The appearance of two lactone carbonyl bands suggested the presence of two isocoumarin rings. Its N.M.R. spectrum ( $\text{CF}_3\text{COOH}$ ) (see Fig. 1) gave signals

aq. filtrate was extracted with ethyl acetate. The two solutions were shaken repeatedly with aq.  $\text{NaHCO}_3$ . Acidification of the combined aq.  $\text{NaHCO}_3$  washings of both the solutions gave 4-carboxy-7-methoxy-3-methylisocoumarin that crystallised from water as needles m.p. and mixed m.p. with the authentic specimen<sup>1</sup> 210–11°, yield 0.07 g.

Concentration of the benzene solution gave the substance A, i.e., 4-(7-methoxyisocoumarin-3-yl)-7-methoxy-3-methylisocoumarin (V) that crystallised from benzene-ethanol mixture as pale yellow needles, m.p. and mixed m.p. with the earlier

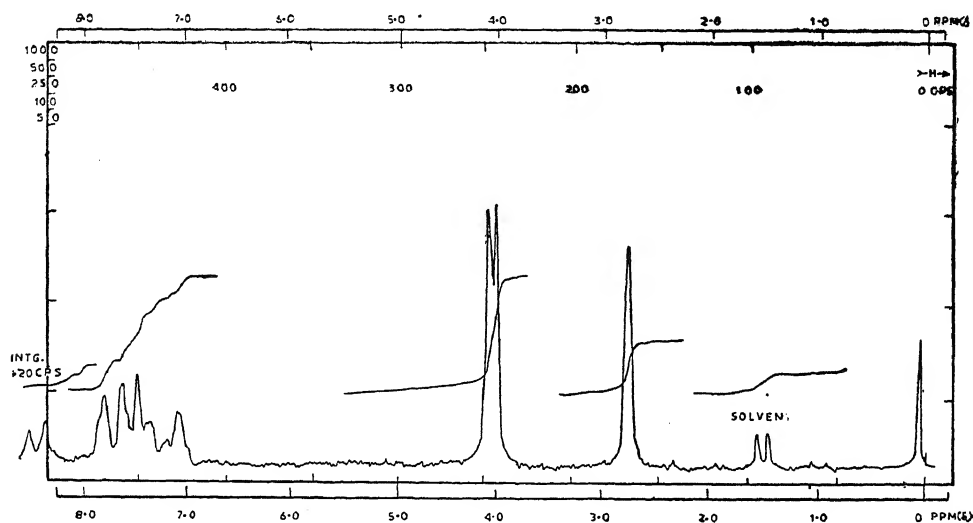


Fig. 1

at  $\delta$  2.7 (3 H, s) for  $=\text{C}-\text{Me}$ , 3.96 (3 H, s) and 4.02 (3 H, s) for two OMe, 7.02 (1 H, s) for the proton (a), 8.37 (1 H, d, J, 9 Hz) and signals between 7.25 and 7.85 (5 H, a sort of m) for the aromatic protons. The proton doublet at  $\delta$  8.37 seems to be due to the proton (b) whose downfield value must be due to steric congestion caused by bulky isocoumarinyl ring in position 4.

#### Experimental

*N,N*-Dimethylaniline catalysed condensation of 4-methoxyhomophthalic acid<sup>3</sup> (I) with acetic anhydride.—A mixture of I (2 g), acetic anhydride (4 ml) and anhydrous N, N-dimethylaniline (2.5 ml) was refluxed for 2 hr and then poured in dilute aq. HCl and left aside at room temperature overnight. The sticky solid that separated was taken in hot benzene with little ethanol and the

sample<sup>1</sup> (substance A) 234–35°, yield 0.18 (Found: C, 69.1, H, 4.5;  $\text{C}_{21}\text{H}_{16}\text{O}_6$  requires C, 69.2; H, 4.4).

The authors thank Professor T. R. Govindachari (Ciba Research Centre) and Professor S. C. Bhattacharyya (I.I.T., Bombay) for N.M.R. and I.R. spectra.

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# TRITIATION OF ORGANIC COMPOUNDS USING TRITIATED WATER\*

Isotope exchange methods have been used for the determinations of active hydrogen in many organic compounds<sup>1-3</sup>. Labelling of compounds by this technique enables one to obtain high specific activities, especially in the labile group. Tritium gas exposure method of obtaining labelled compounds may pose problems of radiation decomposition of the substances and is of use if both labile and bound tritiation is desired. The isotope exchange procedures generally make use of the isotope exchange of active hydrogen in the sample with that in hydroxyl tritiated alcohols<sup>2,3</sup> and tritiated water<sup>4</sup> or of the reaction of active hydrogen with tritiated lithium aluminium hydride<sup>5</sup>. After equilibration the compound of interest can be separated from tritiated alcohol or water, either by vacuum distillation or by specific solvent extraction procedures and analysed by direct liquid scintillation counting of the sample or by counting the water obtained by burning the sample in an atmosphere of oxygen. In the present work, results of the determination of active hydrogen in some fatty acids and alcohols using tritiated water and carbohydrates using tritiated ethanol are reported.

**Materials and Method.**—4 ml of tritiated water ( $0.5 \mu\text{C}/\text{ml}$ ) was mixed with 1–5 ml or 0.2–1.0 g of fatty acids or alcohols and kept for 30 minutes for equilibration. The fatty acid was then extracted into 5 ml benzene. If the acid is a miscible one like *n*-butyric acid, water phase was once again extracted with 5 ml benzene to remove traces of acid. The organic phase was shaken with 5 gm of freshly dehydrated anhydrous sodium sulphate to remove traces of water and kept in a phosphorus pentoxide desiccator bath (30 min) prior to counting.

For the study of carbohydrates, tritiated ethanol was prepared as follows and used. A 5 ml of ( $20 \mu\text{C}/\text{ml}$ ) tritiated water was added to 100 ml of ethanol and the mixture kept for an hour for equilibration. The mixture was distilled at  $78^\circ\text{C}$ , the distillate was kept overnight with activated molecular sieve spheres of the type 13 X (60–80 mesh) (City Chemical Corporation, USA) and then redistilled at  $78^\circ\text{C}$ . 4 ml of this tritiated ethanol ( $0.4 \mu\text{C}/\text{ml}$ ) was treated with 0.4–1.0 g of carbohydrates and kept for equilibration in cold or refluxed. Solvent was decanted and the final traces of solvent were removed from the carbohydrates by vacuum distillation.

A scintillator solution containing 6.5 g PPO, 0.13 g POPOP and 100 g naphthalene in one litre of dioxan was prepared. 0.5 ml of the organic phase was added to 10 ml of the scintillator and

counted. The labile exchange of tritium in the sample was determined by comparing with 0.5 ml aqueous phase activity. Suitable quenching corrections were made to both the phases. Pure benzene extracted with 4 ml tritiated water was used as the blank in these determinations.

The samples were counted in a manually operated liquid scintillation spectrometer constructed by Electronics Group, BARC (Model 206 A). The counter consists of double photomultiplier system aligned in line with the sample and coupled to pre-amplifiers and high voltage units. The instrument is designed to function at room temperatures.

**Results.**—Quenching effects due to various acids and alcohols were studied and are represented in Figs. 1 and 2. Fatty acid or alcohol (5 ml) was

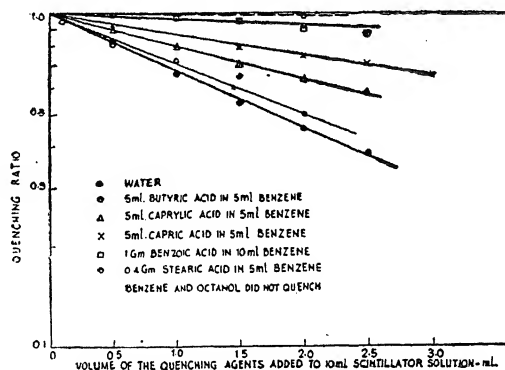


FIG. 1. Quenching of fatty acids.

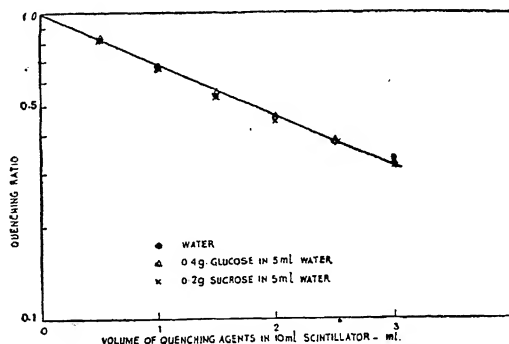


FIG. 2. Quenching of carbohydrates.

mixed with 5 ml benzene and different volumes of this mixture were added to 10 ml of scintillator spiked with known amount of tritiated water. The figures indicate that the hydrocarbons and alcohols do not quench in the concentration ranges studied. A classification was made of the quenching capacity of these reagents on the basis of water quenching. Though from the figure it appears that the quenching due to fatty acids is small compared to that of water, the  $q$  (quenching constant) values obtained from the



TABLE I

Classification of quenching materials (on water quenching basis)

No quenching	Mild quenching	Strong quenching
Benzene	Stearic acid	<i>n</i> -Butyric acid
Di-isopropyl ether	<i>n</i> -Capric acid	<i>n</i> -Caprylic acid
<i>n</i> -Octanol	Benzoic acid	
<i>n</i> -Hexanol		
Sucrose		
Glucose		

the volume of the aqueous phase. Even in that case the error is found to be within 10%–15%.

Sucrose does not exchange appreciably when treated with tritiated ethanol, either in cold or hot; probably due to the OH groups being attached to the ring structure. Glucose also behaves similarly in cold tritiated ethanol but when refluxed with ethanol, it indicated a possibility of one exchangeable hydrogen atom.

The amount of sample required for the determination of active hydrogen depends on. (i) the

TABLE II

Exchange of tritium in some organic compounds

Compound	Solvent used for tritiation	Separation procedure	cpm/g compound	Molar activity ratio
			cpm/g solvent	
Butyric acid	.. HTO	Benzene extraction	0.114	1.11
Caprylic acid	.. HTO	"	0.065	1.04
"	.. HTO	"	0.055	1.05
Stearic acid	.. HTO	"	0.033	1.04
Benzoic acid	.. HTO	"	0.062	0.84
<i>n</i> -Octanol	.. HTO	"	0.070	1.01
<i>n</i> -Hexanol	.. HTO	"	0.096	1.09
Benzene	.. HTO	"	0.001	0.009
<i>n</i> -Di-isopropyl ether	.. HTO	"	0.006	0.06
Sucrose*	.. C <sub>2</sub> H <sub>5</sub> OT	Vacuum distillation	0.034	0.25
Glucose*	.. C <sub>2</sub> H <sub>5</sub> OT	"	0.272	1.07
Glucose	.. C <sub>2</sub> H <sub>5</sub> OT in cold	"	0.006	0.02

\* Refluxed with ethanol for 30 min.

equation  $S = S_0 e^{-\lambda t}$  ( $S_0$  = true count rate,  $S$  = quenched count rate) when the respective concentrations are used (gm/ml) indicate that some are strong quenchers than that of water (6.44 for caprylic acid, 9.1 for butyric acid compared to 4.3 for water<sup>6</sup>).

Tritium exchange technique is useful for the determination of low concentration of active hydrogen present as functional hydroxyl or carboxylic groups in the organic compounds. The ratio of tritium exchanged per gram acid or alcohol to that of tritium present in the aqueous phase per gram is given in Table II along with the molar activity ratio.

The exchange of active hydrogen atoms in the fatty acids and alcohols studied with tritium is found to be very rapid. There was no difference in the ratio, for half an hour to three day exchange periods. The experiments conducted with different amounts of acids and alcohols showed no difference in the activity ratio. This method of determining active hydrogen does not give rise to any difficulties for immiscible systems whereas for butyric acid which is miscible with water, the ratio was found to be slightly higher. This is due to the difficulty in purifying the aqueous phase from butyric acid even after double extraction with benzene and it gives lower than the true count rates by enhancing

specific activity of the source tritium material, (ii) the counting method employed, and (iii) the use of special micro-apparatus. If very high specific active tritium water is used, the amount of sample in the experiments described above can be reduced correspondingly. In this connection the tritiation of organic compounds using only a few milligrams of sample by Giles<sup>3</sup> can be noted. Hence tritiated water can be efficiently used for the active hydrogen determinations.

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# THE SELF-ASSOCIATION CONSTANTS OF METHYLENE BLUE FROM THE CONCENTRATION DEPENDENCE OF THE FLUORESCENCE EFFICIENCY

THOUGH in their pioneering work on the self-association of thionine, Rabinowitch and Epstein<sup>1</sup> measured the concentration quenching of the dye and attributed it to its dimerisation, later workers<sup>2-5</sup> devoted their energies in measuring other properties in their study of the self-association of the dyes. The modern spectrofluorometers are capable of monitoring fluorescence at high dilutions, and so this becomes a useful tool in studying their aggregation at the early stages, as at higher concentrations other factors<sup>6</sup> besides the aggregation contribute towards the fluorescence quenching.

Here we have measured the fluorescence efficiencies of Methylene Blue in the range  $2.3 \times 10^{-6} M$  to  $2.3 \times 10^{-4} M$  to obtain the aggregation constants.

**Experimental.**—Methylene blue, obtained from National Aniline, was used after one recrystallization from ethanol.

Fluorescence measurements were made with a dual monochromator recording spectro-fluorometer. Though the absorption and fluorescence band maxima for Methylene blue are 665 nm and 685 nm respectively, fluorescence was excited at 500 nm and observed at 720 nm, using a path length of 0.25 cm. This was done to ensure that only a small fraction of the exciting light is absorbed by the system and the linearity in the conc. vs. fluorescence curve for the light-emitting species is maintained. Fluorescence was observed at the tail-end of the emission band to avoid errors due to the reabsorption of the fluorescence. Fluorescence was observed from the same side of the cell which received the exciting light.

Preliminary experiments showed that the monomer is the only fluorescing species, as both the fluorescence spectrum as also the fluorescence excitation spectrum remain the same for a dilute and a concentrated solution of the dye. To measure these for the conc. dye, a thin layer of the solution pressed between the wall of the optical cell and a cover glass was used.

All experiments were conducted at  $27^\circ C \pm 1^\circ C$ .

**Results and Discussion.**—Figure 1 shows the relative fluorescence yield of Methylene blue as a function of concentration. At higher concentrations, uncertainty is introduced due to the reabsorption of fluorescence.

The monomer concentration of the dye is calculated from the relation  $b/B = \phi/\phi_0$  where  $b$  and  $B$  are the monomeric and total dye concentration, while  $\phi$  and  $\phi_0$  are the relative fluorescence

yields of the experimental solution and the extrapolated value to infinite dilution.

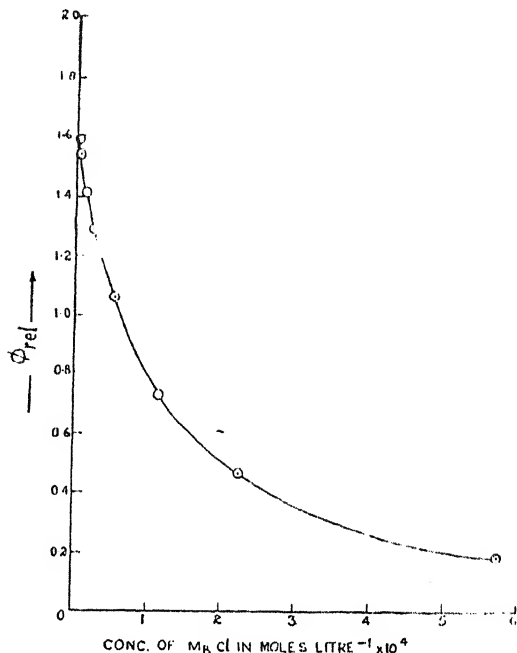


FIG. 1. Change in the fluorescence yield of Methylene blue with concentration.

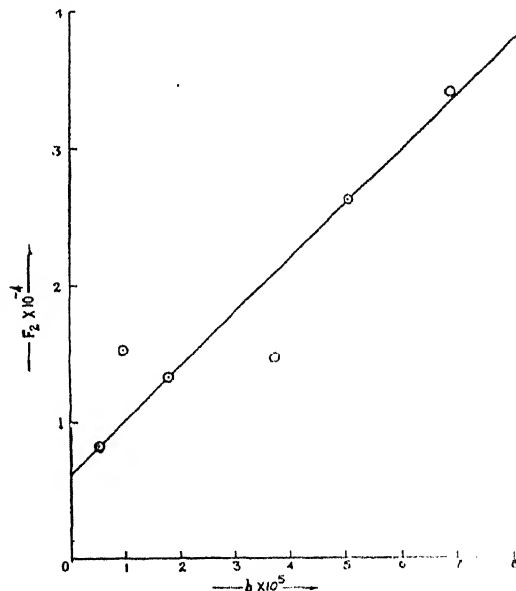


FIG. 2.  $F_2$  vs  $b$  plot.

The data were treated by the method already described<sup>3</sup>, by calculating,  $F_2$  where  $F_2 = \frac{B}{b^2} = 2K_2 + 3K_2K_3b + \dots (1)$ .  $K_2$  and  $K_3$  are the

dimerisation and the trimerisation constants respectively.

In Fig. 2,  $F_2$  has been plotted against  $b$ . A straight line can be drawn through the points, showing that in this range of dye concentration, monomer  $\rightleftharpoons$  dimer  $\rightleftharpoons$  trimer equilibria can adequately describe the data. The  $K_2$  and  $K_3$  calculated from our curve are  $3.0 \times 10^3$  litre mole $^{-1}$  and  $4.4 \times 10^3$  litre mole $^{-1}$  respectively.

These results indicate that fluorescence measurements may be valuable in the study of the self-association of the dyes, especially in dilute solutions. The dimerisation and the trimerisation constants obtained by this method for Methylene blue match well within experimental limitations with the values obtained by the iso-extraction<sup>3</sup> procedure, and the spectrophotometric method<sup>1,4,5</sup>.

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### FOLATES OF *LACTOBACILLUS CASEI*, *STREPTOCOCCUS FAECALIS* AND *PEDIOCOCCUS CEREVISIAE*

THE transport phenomenon for folic acid is evident from a number of reports<sup>1,2</sup> and the work of Wood and Hitchings<sup>3</sup> demonstrated its energy dependence. The existence of multiple forms<sup>4,5</sup> of folic acid in cells were assessed by chromatographic separation and by differential microbiological assays with *Lactobacillus casei*, *Streptococcus faecalis* R and *Pediococcus cerevisiae*<sup>6</sup>. The differential response depends on the nature of the conjugation of the folate derivatives. An analysis of the folate profiles of the assay organisms was attempted in the present studies to understand the reasons for this differential response.

*L. casei*, *S. faecalis* R and *P. cerevisiae* were grown in shake flask cultures at 30°C for 18 hr in a fortified medium containing 2% glucose, 0.5% yeast extract, 0.5% peptone, 0.5% proteolysed liver extract and 0.1% sodium acetate, at 6.8 pH. The organisms were cultured in 200 ml aliquots of medium dispensed in 500 ml Erlenmeyer flasks. The organism was harvested by centrifugation at 0° and washed free of adhering medium. The cell suspension in 1% potassium ascorbate was heated to 75°C for 30 minutes and centrifuged<sup>7</sup>. The folates were separated by chromatography on DEAE cellulose<sup>8</sup> column and assayed according to the method of Teply and Elvehjem<sup>9</sup> using leucovorin (Lederle) as the standard.

It is seen from the present studies, that the differential response of the test organisms to folate compounds is reflected not only in the contents of the folic acid but also in the nature of the folates. From the data (Table I) on the folic acid content

TABLE I  
Cellular folate contents of *L. casei*, *S. faecalis* R and *P. cerevisiae*

Organisms	Folate activity $\mu$ g per mg dry wt of cells as assayed with		
	<i>L. casei</i>	<i>S. faecalis</i> R	<i>P. cerevisiae</i>
<i>L. casei</i>	1.8	0.84	0.79
<i>S. faecalis</i> R	4.2	0.53	0.48
<i>P. cerevisiae</i>	3.4	0.62	0.52

of the cells, it is evident that although the folic acid content of *L. casei* cells is comparatively low, the proportion of *S. faecalis* R active folates is higher in *L. casei* than the other two organisms. The converse was found to be true with *S. faecalis* R and *P. cerevisiae* where folates mostly constituted *L. casei* activity. Studies on the separation of folates on column (Fig. 1) showed the presence

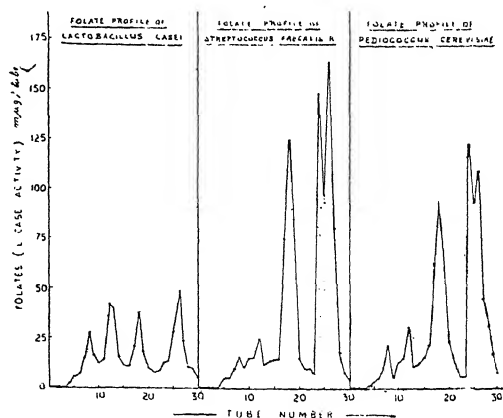


Fig. 1

of N<sup>5</sup> methyl tetrahydrofolic acid (N<sup>5</sup>-methyl THFA) in the fraction corresponding to tube No. 13. This was more predominant in *L. casei* than in *S. faecalis* R and *P. cerevisiae* cells. The folate fractions eluted out in tube Nos. 18, 24 and 26 constitute the folate polyglutamates on the basis of response to the test organisms. Although the substituent units and the state of reduction of these is not ascertained, its presence in the cell raises some pertinent questions. It is probable that *S. faecalis* R and *P. cerevisiae* are not able to utilise these molecules due to the lack of enzymes to breakdown higher molecular weight derivatives of folic acid. However, the report of Goulian and Beck<sup>10</sup> points to the ability of cells to store large amounts of folates which are made available as the need arises. Once the folate monoglutamates are taken by the cells they could be converted to folate polyglutamates for storage or use in specific enzymic reactions<sup>11</sup>. The high *L. casei* activity noted in *S. faecalis* R and *P. cerevisiae* could be a result of this.

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#### SOME STUDIES ON THE VERMICULITE DEPOSITS OF THIRUPATTUR IN TAMIL NADU

THERE is an extensive occurrence of vermiculite deposits in the Thirupattur Taluk of the North Arcot District of Tamil Nadu and the reserves are estimated to be approximately 2 lakh tonnes as per a survey conducted by the Geology Branch of the Tamil Nadu State. The vermiculite is separated in the mines from accompanying rocks like pyroxenites and graded into four sizes ranging from - 1/8" to + 1". A composite sample of these four

grades of vermiculite from the mines was subjected to some studies including detailed chemical analysis in the laboratory of the Geology Branch.

#### Chemical analysis of Thirupattur vermiculite

Constituents	Per cent	Constituents	Per cent
SiO <sub>2</sub>	30.14	Na <sub>2</sub> O	0.20
Al <sub>2</sub> O <sub>3</sub>	11.64	K <sub>2</sub> O	1.33
Fe <sub>2</sub> O <sub>3</sub>	13.00	"CO <sub>3</sub>	7.23
FeO	0.87	"SO <sub>4</sub>	0.12
TiO <sub>2</sub>	2.27	"CL	nil
MnO	0.13	Cu	0.05
CaO	8.69	Zn	0.01
MgO	12.92	Ni	0.015
		Cr	nil
		V	nil
		Moisture	7.05
		Loss on ignition	11.50

The base exchange capacity of the vermiculite was determined on various sieve fractions (procedure as in reference 1) and the results are as below.

ASTM Sieve mesh No.	Base exchange capacity Meq NH <sub>4</sub> <sup>+</sup> /100 gm sample
-100	78
-200	80
-270	83
-325	88
-400	94

The dehydration characteristics of the vermiculite was studied following the method adopted by Nutting<sup>2</sup>. The sample was subjected to stepwise increase in temperature and measurement at a particular temperature was made till there was no further loss in weight. The dehydration curve is shown in Fig. 1. A point to note is, that due to the presence of carbonates, the loss in weight is not due to water alone.

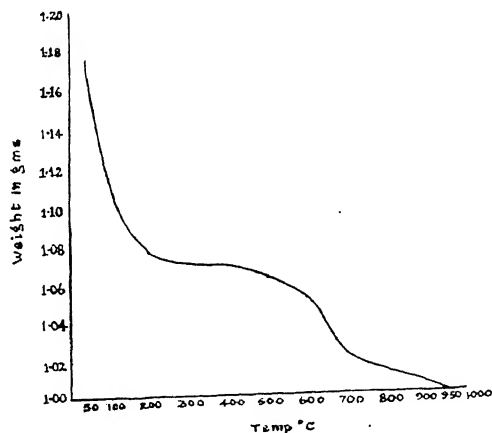


FIG. 1. Dehydration curve. The weight at 950° C is taken as the base weight in plotting the curve.

The Thirupattur vermiculite is being commercially exploited and an exfoliation plant set up at Ambattur by the Government of Tamil Nadu is manufacturing exfoliated vermiculite.

The Department of Industries and Commerce is thanked for offering facilities for conducting the above work. My thanks are due to the Additional State Geologist for the interest shown and to Thiru M. Jayakumar and Thiru C. R. Raja Adithan, Senior Chemists of the laboratory, for assisting in the work.

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#### SEX SPECIFIC PROTEINS IN THE BEETLE *AULACOPHORA FOVEICOLLIS* LUC. (COLEOPTERA, CHRYSOMELIDAE)

USING polyacrylamide gel disc electrophoresis, haemolymph proteins have been fractionated. Presence of 3 female specific proteins and a human serum albumin like protein has been observed.

In recent years considerable attention has been paid to the characterisation of haemolymph proteins (Florkin and Jeuniaux, 1964; Wyatt, 1961; Chen 1966). It has been reported that the haemolymph of female insects contains extra protein/proteins which are not detected in the haemolymph of males. The presence of these female sex specific protein/proteins have been reported in a number of insects such as *Periplaneta* (Adiyodi and Nayer, 1966), *Hyalophora* (Stephen and Steinhauer, 1957), *Schistocerca gregaria* (Kulkarni and Mehrotra, 1970), *Leucophaea* (Engelmann and Penney, 1966), *Leptinotarsa decemlineata* (De Loof and De Wilde, 1970), *Musca* (Bodnaryk and Morrison, 1968), *Rhodnius* (Coles, 1965), *Triatoma infestans* (Perassi, 1973) and *Sarcophaga bullata* (Wilkens, 1969).

Despite the increasing interest very little work has been done on the haemolymph proteins in Coleoptera. Here an attempt is made (i) to find out the differences in the haemolymph protein patterns of male and female *Aulacophora* and (ii) to locate, if any, the female sex specific protein/proteins in this insect.

The insects used were collected locally from the fields in Saugar, M.P. (India). Haemolymph from

adults of both sexes was obtained by making a small puncture at the base of the coxa of the first leg. A drop of haemolymph, so exuded, was taken and used immediately for the purpose of electrophoresis on polyacrylamide gel.

The polyacrylamide gel disc electrophoresis method adopted was similar to that described by Davis (1964). Gel tubes were taken out and stained for 2 to 3 hours in 1% (W/V) Naphthalene black in 7% acetic acid followed by destaining at room temperature in 7% acetic acid till the stain is cleared out from the gel, excepting the protein fractions. Rm value was calculated by the method of Kulkarni and Mehrotra (1970).

By using polyacrylamide gel disc electrophoresis, in all 8 different protein fractions have been detected in the haemolymph of the adult female *Aulacophora*. In adult male *Aulacophora* only 6 protein fractions could be observed (Fig. 1).

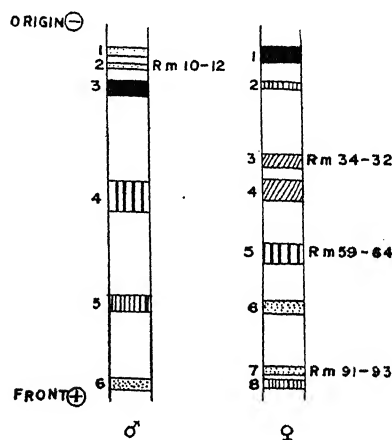


FIG. 1. Haemolymph proteins *Aulacophora foveicollis* Luc.

Haemolymph protein fractions 1 and 2 in females, and fractions 3 and 5 in males are deeply stained with the protein dye, therefore, they are considered as the major protein components in both the sexes respectively.

Working on the insects of different orders many workers have reported the presence of sex specific protein. As far as the author is aware the presence of sex specific protein in the insect belonging to Coleoptera has been reported for only one insect, i.e., *Leptinotarsa decemlineata* (De Loof and De Wilde, 1970).

In *Aulacophora* three protein fractions, i.e., fraction 3 (Rm 34-38), 5 (Rm 59-64) and 7 (Rm 91-93) are found only in females. Proteins with the similar Rm values are not noted in the haemolymph from males. These proteins are named as

"female sex specific proteins". In the haemolymph from males, protein fraction 2 (Rm 10-12) is an extra protein which could not be detected in the haemolymph protein fraction of females. This protein, like the female sex specific proteins, can be denoted as "Male sex specific protein". It might be playing some role in the physiology of male *Aulacophora*. It is also probable that the 1 band present in the females (which is always darkly stained with the dye) is a heterogeneous band containing more than one protein. This heterogeneous band has low concentration in male as compared to female, hence the two bands could easily be detected in the haemolymph of adult male *Aulacophora*.

Rm values almost comparable to that of human serum albumin is shown by band 6 in males and fraction 8 in females. The possibility of human serum albumin like protein or "Insect albumin" is still controversial. The human serum albumin like protein could not be observed in *Phormia* (Chen and Levenbook, 1966) and other insects, while the same has been reported in the haemolymph of 7 different insect species tested by starch gel electrophoresis (Whittaker and West, 1962), in honey bee larva (Bishop *et al.*, 1925), and in *Schistocerca* (Kulkarni and Mehrotra, 1970).

The changes in the protein picture of haemolymph at different developmental stages would be published elsewhere.

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## A SIMPLE SHALLOW-WATER SAMPLER FOR RECORDING TEMPERATURE

TAKING water temperature at depths is difficult in the absence of a reversing thermometer or a Pettersson sampler. The problem becomes more acute in shallow waters of a few feet in depth where the reversing thermometer cannot be operated. The authors have developed for the purpose a shallow-water sampler which could be used upto 50 feet of depth (Fig. 1).

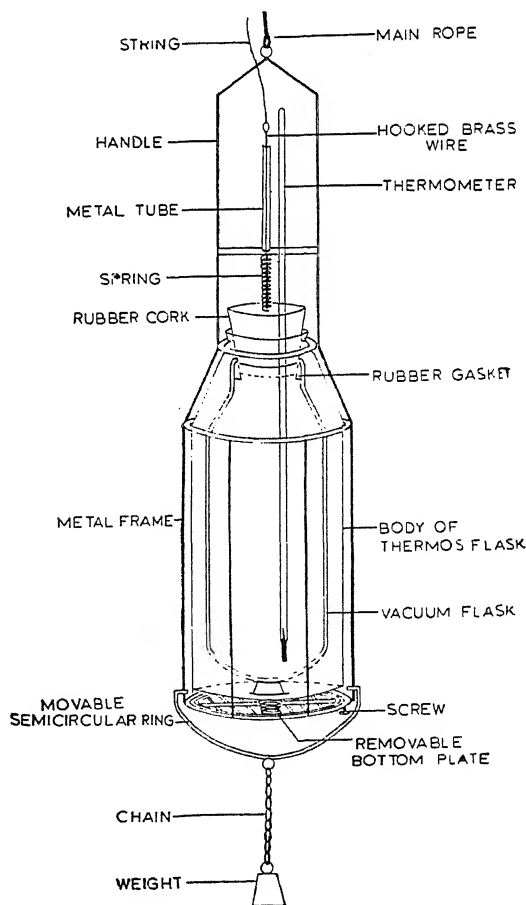


FIG. 1

The sampler consists of a weighted steel-frame into which is fitted a vacuum flask of the capacity 425 ml. The flask has a narrow mouth with a rubber gasket. A suitably ground rubber cork is fitted in the mouth. Through the centre of the cork is passed a close-hooked brass wire of 5 mm thickness. At the other side of the cork the wire is fixed by a nut-bolt. The cork is pressed in position by a brass spring fixed in between the frame and the cork. Outside the steel frame, the brass wire is passed through a metal tube in order that it does not damage the thermometer and also moves smoothly while opening and closing the bottle. To the lower end of the frame of the sampler is fixed a movable semicircular ring with a chain and weight. This prevents the accidental striking of the sampler with the bottom of the pond or lake and also ensures its smooth vertical descent in the water. The frame has a removable bottom plate so that the inner flask can be removed for cleaning or replacement. The handle of the sampler is made long to avoid touching of the thermometer or interfering with the reading. A 50°C thermometer is passed through the cork and fixed in position about half an inch above the bottom of the inner container of the vacuum flask.

During operation, the sampler is lowered in water by the main rope and opened at desired depth by pulling the string attached to the hooked brass wire. The sampler gets filled up within 15–20 seconds. On the release of the string, the sampler is closed due to the spring and there is no mixing of sample with the water at the higher levels while the sampler is being hauled. The temperature of the water can be read after hauling out the sampler.

The sampler has repeatedly been used upto the depth of 40 feet in ponds, lakes and wells. It was found that the temperature does not change even 0.1°C till 20 minutes after hauling of the sampler. The sampler was used along with sophisticated water-samplers to compare its efficiency and it was noticed that it is in no way inferior for the purpose it is designed.

The water sample collected by the sampler cannot be used for oxygen estimation because of the bubbling during filling of the sampler under water. However, the sample can be used for the estimations of other chemical constituents.

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#### ON THE CULTIVATION OF *GRACILARIA* *EDULIS* IN THE NEAR SHORE AREAS AROUND MANDAPAM

AGAR-YIELDING algae are restricted to certain parts of the east and west coasts of India<sup>1</sup> and the agar industry may require more raw material than the crop available in natural habitats. In order to augment the production of these agarophytes detailed studies must be undertaken in our country to transplant the important species to other suitable localities and to cultivate them on a large scale. With the development of the seaweed industry in our country some culture experiments were made on *Gracilaria edulis* (Gmelin) Silva and *Gracilaria corticata* J. Agardh<sup>1,2</sup>. Recently, Raju and Thomas<sup>3</sup> cultured *G. edulis* in a sandy lagoon on the eastern side of the Krusadai Island, where the water is calm for most part of the year. To study the possibility of cultivation of *G. edulis* in open shore environment field experiments were conducted in the Gulf of Mannar near the Jetty of the Regional Centre, CMFRI and the present report summarises the information obtained for four months from January to April, 1973.

Coir net frames of 4 × 2 m size were used in the present study. Fragments of *G. edulis* (about 4.0 cm long) taken from the apical parts of the plant were used as 'seed' material and they were inserted in the twists of the coir rope (Fig. 1 a). About 2.5 kg of material was used for seeding one frame. The first frame with seed material was tied to the poles planted near the jetty on 12th January 1973 and the second one on 19th January, 1973. These frames were horizontally placed (Fig. 1 b) and tied to poles with coir rope at subtidal level to keep them in submerged condition throughout the period of this investigation. Unlike in the rope culture method<sup>3</sup>, these horizontally placed coir nets would give support to plants when fully grown and also help in minimizing the breakage and removal of fronds by wave action and water currents.

Harvesting of *G. edulis* was done by cutting the plants with sickles, leaving the basal parts on the frames for further regeneration. The first and second frames were harvested on 2nd and 9th April, 1973 respectively and the fresh and dry weights of the material harvested were determined.

Fragments of *G. edulis* inserted in the second and third weeks of January, 1973 could grow to maximum size within three months. Data obtained on the standing crop are given in Table I. As may be seen, the density of the crop (Fresh weight/m<sup>2</sup> area of the frame) varied on the two frames and on an average 4.4 kg fresh weed was obtained per square metre area of the coir net. For comparison, data

TABLE I

Fresh and dry weights and rate of production of *G. edulis* on culture frames

Frame No.	Date of commencement	Date of Harvest	Growth period (days)	Fresh weight (gm)	Dry weight (gm)	Density (gm Fr.wt./ m <sup>2</sup> )	Rate of production (gm/day/m <sup>2</sup> )
1	12-1-1973	2-4-1973	80	25,327	4,230	3,442	43.0
2	19-1-1973	9-4-1973	80	43,219	6,830	5,402	67.5
Mean				34,273	5,530	4,422	55.25

collected on *G. edulis*, in sample surveys conducted in the Gulf of Mannar and Palk Bay regions near Mandapam,<sup>2,4</sup> are given below:

	% Frequency	Density (kg/m <sup>2</sup> )
Gulf of Mannar ..	7.04	0.019
Palk Bay ..	11.38	0.034

However, from the results obtained in the present study the rate of production appears to be more in open shore environment (Table I).

The growth cycle of *G. edulis* occurring in the shallow sublittoral region near Rameswaram was studied and in the natural environment this alga took 4 to 5 months time to grow to maximum length, with a growth rate of 1.34 mm/day during the primary growth season<sup>5</sup>. On the culture frames plants of *G. edulis* could grow to harvestable size within three months, with an average rate of 55.25 gm/m<sup>2</sup>/day (Table I). These preliminary observations indicate that the near shore areas in the vicinity of Mandapam are also suitable for the cultivation of *G. edulis* and using coir nets good yield can be obtained during the calm seasons of the year either on the Gulf of Mannar side or on the Palk Bay side of the coastline.

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#### DICHLORVOS (DDVP) INDUCED BREAKS IN THE SALIVARY GLAND CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

DICHLORVOS (Dimethyl-2, 2-dichlorovinyl phosphate) is a widely used organophosphorus pesticide and is effective against a wide variety of insects. As such, it is an active ingredient in some commonly used formulations like "NUVAN" and slow-release PVC strips "VAPONA". DDVP has been shown to exhibit alkylating properties<sup>1-3</sup>. Since many of



FIG. 1. Cultivation of *Gracilaria edulis*: (A) part of the coir net with seed material; (B) inshore area showing the culture frame.

*G. edulis* was found in 7 to 11% of the quadrats sampled; its density in the natural habitats was very much lower than that obtained in culture experiments (Table I).

In the experiments conducted in the lagoon area by Raju and Thomas<sup>3</sup> three harvests were made at the end of five, eight and ten and half months and an annual yield of about 3.5 kg has been reported per one metre length of the rope. Since the method employed is different, it may not be possible to compare the yield and rate of production of *G. edulis* in the lagoon and inshore areas.



the known chemical mutagens are alkylating agents, it was considered worthwhile to study the possible mutagenic effects of DDVP. Seven different concentrations (50 ppm to 1 ppm) of commercial "NUVAN" (100 EC sold by Ciba Ltd.) were prepared. One ml. solution of each concentration was mixed with 10 gm of food in a vial and 15-20 female *Drosophila* flies were kept in each vial. Salivary gland chromosomes of fully grown (third instar) larvae from these flies were examined for chromosomal aberrations.

It was observed that even low concentrations of DDVP were highly toxic. No eggs were laid by the flies when DDVP was given in concentrations above 10 ppm. At concentrations lower than 10 ppm some egg laying was observed but sufficient number of larvae could not be obtained. Even at 1 ppm concentration, 45% survival was recorded, as compared with the controls. The observations on chromosomal aberrations induced are given in Table I.

TABLE I  
Chromosomal aberrations induced by Dichlorvos

Treatment	No. of cells studied	No. of cells showing aberrations	No. of cells with	
			Inversions	Deletions
Control	107	00	00	00
1 ppm	108	11	10	1
DDVP		(10%)	(9%)	(1%)

It is seen that 10% of all the cells studied at 1 ppm concentration showed chromosomal aberrations while in control, no such abnormality was recorded. Inversions were frequent and only one deletion was observed. It was not possible to assess precisely the preferential effect of DDVP on different chromosomes but inversions were more frequently observed in the X chromosome and chromosome III. The single deletion observed was in the left arm of chromosome II.

These observations show that DDVP is a mutagenic agent in *Drosophila*. Sax and Sax<sup>4</sup> have also reported that when onion seeds are allowed to germinate in the air contaminated by "Vapona" strips, upto 13.4% of the root-tip cells show chromosomal aberrations. The residues of this insecticide have been found in the air as well as in the food cooked in houses using "Vapona" strips, even 70-120 days after placing these strips<sup>5,6</sup>. The widespread use of DDVP and other insecticides leaving a variety of residues may, therefore, pose a serious problem and threaten the genetic health of coming generations. In recent years there has been increasing concern about the mutagenic potential of various chemical agents and drugs now

found in our environment. The ability of a chemical to produce mutations in *Drosophila* or other test systems may not always mean a similar effect in human beings but the potential hazard cannot be ruled out. A large number of environmental agents have been screened for their mutagenic properties, using bacterial and other test systems<sup>7,8</sup>. These tests can be used to establish priorities for a thorough testing in mammalian systems to evaluate the possible mutagenic effects for man.

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#### EFFECT OF ETHYL HYDROGEN 1-PROPYL PHOSPHONATE ON THE ONTOGENY AND ORIENTATION OF STOMATA OF *VICIA FABA* L.

NIAGARA (Ethyl hydrogen 1-propyl phosphonate) has been reported<sup>1</sup> to interfere and retard the growth of a number of herbaceous and woody plants. It particularly brings about changes in the foliar anatomy since it is absorbed by both root and foliage of the herbaceous plants, readily. Observations on the effects of Niagara on the leaf ontogeny of *Vicia faba* L. with particular reference to the epidermis are presented in this paper.

*Vicia faba* seedlings raised 45 cm apart in the field were sprayed with an aqueous solution of the Niagara at 6-8-leaf stage. The concentrations used were 100, 500, 1000, 5000, and 10,000 ppm (+ 0.02% tween being used as surfactant). Twelve plants were used for each treatment. A set of three plants from each row remained without spray and was maintained as control.

The leaves from the treated plants of each concentration as well as those from the control were collected 10 days after the spray and fixed in FAA. The second round of collection was performed one month after the treatment. The peels of three leaves under each concentration

were stained with 1% safranin, mounted in glycerine jelly and studied.

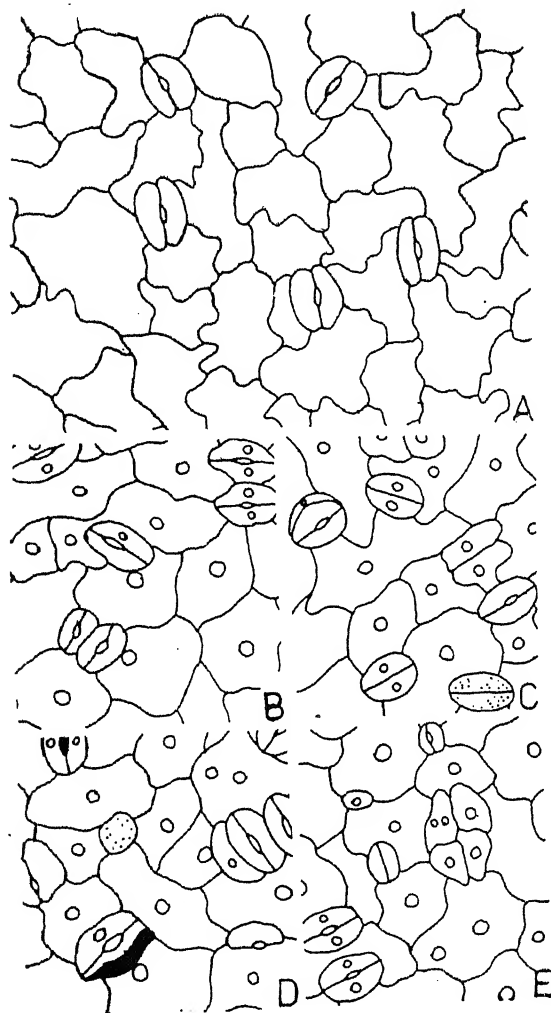


FIG. 1. Effect of Niagara on the stomatal ontogeny and morphology of *Vicia faba*. A Orientation and structure of the stomata in the epidermal cells in the untreated leaves; each stoma has two guard cells and is irregularly oriented; the epidermal cells surrounding it are sinuous-walled,  $\times 380$ . B. Epidermal peel from Niagara-treated leaves showing contiguous stomata, persistent meristemoids, and division of the epidermal cells,  $\times 380$ . C. Another view of the same with incomplete development of the guard cells, division of epidermal cell nucleus without the wall septation, persistent meristemoids, and division of epidermal cells,  $\times 380$ . D, E. A portion of the leaf epidermis from treated leaves; mark the straight to feebly arched epidermal cells, comparatively smaller size of the stomata, division of the guard cells, incomplete development of the guard cells, and the division of the epidermal cell nucleus without wall septation,  $\times 380$ .

Observations were recorded 10 days after the spray. The controls showed irregular orientation of stomata, with two guard cells; the epidermal cells themselves had a highly wavy outline (Fig. 1 A). The stomata developed perigenously and are devoid of subsidiary cells (asakshoshik type). The plants treated with Niagara showed considerable modifications of the epidermal tissue as compared to the controls. The number of stomata increased and also the number of epidermal cells per unit area (Table I). The size

TABLE I  
Stomatal frequency in the control and treated plants of *Vicia faba* (per. sq. mm)

Control	100 ppm	500 ppm	1,000 ppm	5,000 ppm	10,000 ppm
16.5	16.9	18.0	19.6	20.1	50.7

of stomata was significantly reduced. The nuclei showed divisions in some cells without being accompanied by septa formation, and the walls of the epidermal cells become more or less straightened to arched with reduction in their size. Frequently, the divisions of the entire epidermal cells were also noticed (Fig. 1 B). In Fig. 1 B, C are seen contiguous stomata, incomplete development of the guard cells as well as the lack of differentiation of the meristemoids (the persistent initials or meristemoids). Figure 1 D brings out some interesting abnormalities caused due to the effect of the chemical which include a smaller stoma, the meristemoid, and an enlarged stoma with divisions in both the guard cells, thus making a 'tetrad-like' structure. It also shows the development of a single guard cell and the absence of the other counterpart. Additional anomalies seen in Fig. 1 E are contiguous stomata, two septate, stomata-like structures and divided epidermal cells. Table II summarizes the numerical values of the various types of anomalies caused by the treatment of plants with different concentrations of Niagara.

The control as well as the treated plants were allowed to grow further to ascertain variations in their growth patterns. Interestingly enough, the epidermis of treated plants showed 'proembryo-like' bodies as well as contiguous stomata (even up to 6 of them being placed adjacent to each other), in much increased percentage, after a lapse of one month (Fig. 2). These originate by repeated divisions in the epidermal cells or the guard cell initials. Both these as well as the epidermal cells also possessed numerous starch grains.

It is evident, therefore, that like the other chemicals, Niagara 'influences' the leaves in many

TABLE II  
Responses of leaf epidermis of *Vicia faba* to Niagara

Types of anomalies %	Niagara concentrations used				
	100 ppm	500 ppm	1,000 ppm	5,000 ppm	10,000 ppm
Contiguous stomata	0	0	18.9	20.7	24.2
Incomplete dev. of guard cells	0	0	0	9.9	1.1
Degeneration of guard cells	0	26.3	35.6	8.3	0
Division of epi. cells	0	0	20.0	40.7	10.9
Division of epi. cells without wall formation	0	9.6	0	0	0
Persistent meristemoids	0	6.8	5.5	2.3	50.3
Division of guard cells	0	0	0	0	10.1
Normal stomata	100	57.3	20.0	18.1	4.4

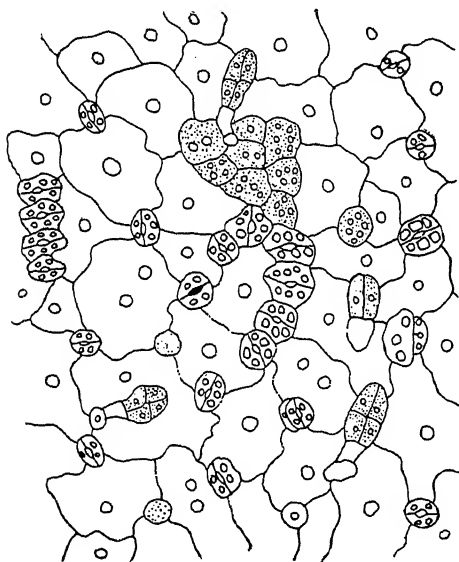


FIG. 2. A portion of the leaf epidermis of *Vicia faba* after one month of Niagara treatment; mark the 'proembryo-like' outgrowth with stalks of hyaline cells,  $\times 380$ .

ways. Cell division is markedly enhanced coupled with their metabolic activity as evidenced by abundance of starch grains in them as well as the guard cells. Significant aspect which also calls for immediate attention of the investigators is to investigate as to how the changed morphology of the stomata influences the respiration and photosynthetic activity of the leaves.

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#### THE CHROMOSOME NUMBERS IN *CONOCEPHALUM CONICUM* (L.) DUM.

THERE are two species of *Conocephalum* reported from India, viz., *C. conicum* (L.) Dum. by Kashyap<sup>2</sup> from Kumaon, Pangi and Outer Himalayas (5000'-8000'), and *C. supradecompositum* (Lindb.) St. from Darjeeling. *C. conicum* grows luxuriantly in humid and shady places near running water channels in Nainital and Ranikhet. In fully developed plant the size of the thallus reaches upto 16 cm in length and stalked carpocephala are found (not observed by Kashyap) in the months of February and March.

Cytological works on *C. conicum* was formerly made by Bolleter<sup>1</sup> and Woodburn<sup>5</sup> who reported  $n=8$ , while Showalter<sup>4</sup>, Lorbeer<sup>3</sup>, etc., reported  $n=9$ . These authors restricted their studies to mitosis only, using either antheridiophore or laboratory cultured apices of male and female plants and chromosome numbers were investigated through microtome sections.

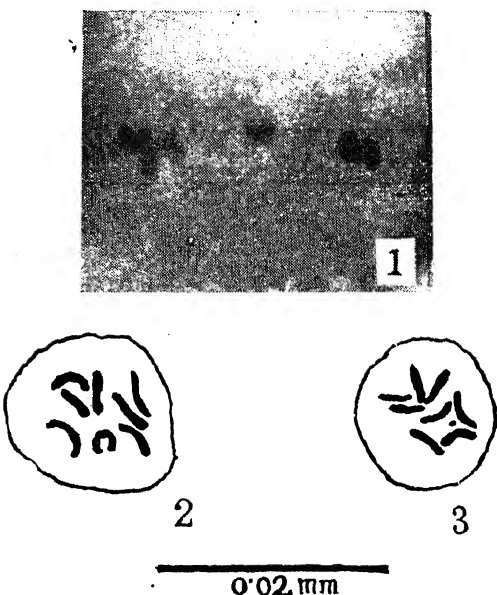
The disparity in above findings encouraged the author to find out the correct number of chromosomes from the material growing at Nainital. In addition to mitosis the present study was also extended to the study of meiosis in young sporophytes.

For mitotic and meiotic studies, material were fixed in a modified form of Carnoy's solution. (The percentage of chloroform was increased from 10% to 20%). Squash preparation was made 2-10 days after fixation, the meiotic chromosomes were stained with acetocarmine. Antheridia were hydrolysed with  $n\text{-HCl}$  at 40° C for fifteen minutes.

Thirty capsules were squashed and fifteen hundred dividing spore mother cells were examined, but in all cases only eight bivalents (Fig. 1) were seen. Similarly twenty antheridia were squashed and

studied. In 95% cells eight mitotic chromosomes were observed, which were rod-like and almost

equal in size (Fig. 2). However, in about 5% cells in addition to eight chromosomes one small chromosome was also found (Fig. 3).



FIGS. 1-3. Fig. 1. Spore mother cell with eight bivalents. Fig. 2. An androcyte cell with eight chromosomes. Fig. 3. An androcyte cell with eight normal and one small fragment of chromosome.

In contrast to the findings of Showalter, who reported nine chromosomes in 80% cases, the occurrence of the 9th chromosome was only 5% in the present study. It appears that in *C. conicum* chromosome number is eight ( $n=8$ ) in their gametophyte, and the ninth chromosome could be a detached part of any of the eight chromosomes. The stable occurrence of eight bivalents in spore mother cells also supports this finding. Thus the haploid chromosome numbers in *C. conicum* is eight ( $n=8$ ).

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## SHORT SCIENTIFIC NOTES

### A New Record of *Astronia macrophylla* Bl. (Melastomataceae) from Great Nicobars and its Phytogeographical Significance

Blume<sup>1</sup> established the genus *Astronia* on the basis of two species, *Astronia spectabilis* Bl. and *A. macrophylla* Bl. from Java. This is a genus of small trees and shrubs bearing polygamo-dioecious flowers with inappendiculate anthers. The genus *Astronia* Bl. (*sensu stricto*) includes about 56 species (excluding *Astronidium* A. Gray) and occurs in Malaya, Sumatra, Borneo, Java, Moluccas, Celebes, New Guinea and Formosa. The Philippine-Papuan region is probably the centre of origin of the genus, since out of the 56 species, this region accounts for about 54 species.

Among these, the widely spread species are *Astronia macrophylla* Bl., *A. smilacifolia* Triana and *A. cumingiana* Vidal. On studying the specimens collected by Rogers from Great Nicobar Island

*C.G. Rogers* 48 was found to match with *Astronia macrophylla*, hitherto reported only from Sumatra, Borneo, Java (Backer and Bakhuizen<sup>2</sup>) Moluccas and Celebes. This new record from Great Nicobar Island extends the known distribution of *A. macrophylla* from Philippines and Indonesia to Andaman and Nicobar group of islands, which form the insular land bridge connecting Sumatra in the South with Arakan mountains of Burma in the north. Since this is the first record for the Indian flora, a brief description is given.

*Astronia macrophylla* Bl., *Bijdr. Fl. Ned. Ind.* No. 17: 1080. 1826; Cogniaux in DC. *Monogr. Phan.* 1096, 1891.

Trees 5-12 m tall; branches brown, furfuraceous. Leaves opposite, elliptic or ovate-elliptic, 20-35 × 8-17 cm, base rounded, apex acuminate, under surface along the nerves furfuraceous, 3-5 plinerved; veins prominent; petiole 5-10 cm long, furfu-

raceous. Inflorescence 10–17 cm long, densely furfuraceous. Bracts lanceolate, 6–10 × 2–2.5 mm; bracteoles linear, 2–3 mm long. Calyx tube campanulate, 2–2.5 mm long, 10-ribbed, furfuraceous; teeth triangular, 0.5 mm long. Petals 5, free concave, obtuse, brown, 2.5–3 mm long. Stamens 10; filaments 2.5 mm long; anthers 1.5–2 mm long. Ovary fully conrescent with the calyx tube; extraovarian chambers absent; styles 2–4 mm long; stigma capitate.

Great Nicobar: C.G. Rogers 48 (CAL); S. Ahmadali 58 (CAL).

I wish to thank the Director, Botanical Survey of India for facilities.

Botanical Survey of India, N. G. NAIR.  
Andaman and Nicobar Circle,  
Port Blair, August 21, 1974.

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#### *Oldenlandia umbellata* L.—An Addition to the Flora of Northern India

J. D. Hooker<sup>1</sup> records the distribution of *Oldenlandia umbellata* L. for India from Western peninsula—from Orissa southwards. Subsequently, the plant has also been recorded by Prain<sup>2</sup> from Bengal. This species has neither been recorded in their lists by Rau<sup>3</sup> or Singh<sup>4</sup> for the Upper Gangetic Plain nor in any "Floras" of Punjab or as a new record for the Punjab plains by Nair and Nair<sup>5</sup>. As far as is known it has not yet been reported from India north of Punjab. Apparently this is a new introduction in the flora of north India. Some plants of this species were collected from a locality in Punjab. The description that follows is from those specimens.

A diffuse, glabrous (very rarely scaberulous on the angles) annual. Stems 4–12.5 cm long, semi-woody and branched at the base, angular. Leaves often fascicled, spreading or recurved, sessile, linear, or almost acicular, 0.4–2.3 × 0.05–0.3 cm, flat with recurved margins, acute. Stipules short with several bristles on the upper margins. Flowers

± 3 mm long, white, 3–7, axillary or umbellate on axillary peduncles, chiefly in the upper axils. Peduncles shorter or longer than the leaves, erect, stout. Pedicels very short. Calyx-tube (in flower) 1 mm long, teeth 4, 1 mm long, subulate, ciliate, nearly equalling the corolla-tube. Corolla glabrous, lobes 4, triangular-oblong. Stamens 4, inserted near the throat of the corolla-tube. Capsule 2 × 2.5 mm, glabrous, crowned with distant calyx-teeth shorter than the capsule. Crown of the capsule not protruded. Seeds many, dark brown, shortly oblong, angled, smooth.

*Specimens examined*: M. Sharma 3224 (PUN).

*Locality*: Samana (alt. 240 m) in Patiala

District of Punjab. Among grasses along a water channel.

*Flowers and Fruits*: November–January.

*Distribution*: Sri Lanka (Ceylon), Burma.

But for its inflorescence the plant resembles much with the prostrate forms of *Oldenlandia corymbosa* L. Many-flowered and umbellate inflorescence immediately separates it from other Indian species of *Oldenlandia* (*sensu str.*) except *O. wightii* Hook. f. which is a perennial herb with scabrid branches and leaves, capitate cymes and long, triangular-lanceolate, pungent calyx-teeth that equal the depressed capsule.

The author is grateful to Prof. S. S. Bir for providing facilities.

Department of Botany, M. SHARMA.  
Punjabi University,  
Patiala (Punjab), August 23, 1974.

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## REVIEWS AND NOTICES OF BOOKS

**Biochemistry—A Problems Approach.** By William B. Wood, John, H. Wilson, Robert, M. Benbow and Leroy, E. Hood. (W. A. Benjamin, Inc., Menlo Park, California, U.S.A.), 1974. Pp. xii + 511. Price \$ 7.95.

This book has been written with a completely new approach for teaching biochemistry and it is radically different from the conventional text books. In every chapter a brief outline of the basic concepts is given, which is followed by a large number of problems and their answers. The problems have been designed in such a way that they not only test the background knowledge of the student but also improve his ability to apply such knowledge in the analysis of biochemical data. But the basic concepts are given in such brief outline that it can be used only as a supplement to standard text books. The book is well organised and well written, and it will be very useful for graduate students.

J. GANGULY.

**Handbook of Vertebrate Pest Control.** By W. D. Fitzwater and Ishwar Prakash. (Indian Council of Agricultural Research, New Delhi), 1973. Pp. 92. Price Rs. 4.50.

Many species of vertebrate animals are pests of agricultural and medical importance. The average farmer or public health worker has very little access to authoritative sources of information on their identification, habits and control. The present handbook is, therefore, a welcome and highly useful compilation which covers a wide range of topics, divided into eighteen chapters, dealing with almost all aspects of the study and control of most major vertebrate pests occurring in India. The handbook is profusely illustrated with line-drawings which, with the accompanying notes on diagnostic morphological characteristics, will help in recognising the different pest species, which is necessary for adopting suitable control methods.

The authors have undoubtedly done an excellent job of condensing a lot of information in such a small handbook. Each chapter is closed with a list of books and journals for supplementary reading, which will be of special value to teachers and research workers. In one of the chapters, first-aid measures against poisoning by various common pesticides are explained in a simple manner. These instructions are often not readily available in rural areas.

Considering the present high cost of printing and paper, the handbook is very reasonably priced.

T. SANKARAN.

**Calculus—A Programmed Text.** By Merriell, (Addison-Wesley Publications, Reading, Massachusetts 01867, USA), 1974. Vol. I: *Techniques and Applications*, Pp. vii + 460 + 17; Vol. II: *Theory*, Pp. xi + 13. Price not given.

Volume I of the Book has five chapters devoted to techniques and three chapters to applications of calculus. The author's justification in adding another book to the numerous existing good books on an ancient subject like Calculus, seems to be his style. It is written in a programmed style and the subject matter is presented in frames which contain blanks to be filled in by the student. This method would replace the passive student response by an active response in the class room.

The techniques and applications are the usual ones. However the author has some relevant interesting remarks to offer on the definitions and proofs. At some stage he states that since it took nearly 150 years for the best mathematical mind to develop a complete understanding of the meaning of limit concept, one should not expect to have on instant comprehension of the definition of a limit.

Volume II has six chapters and deals with Theory. In writing about the proof, the author feels that, like a work of art, the final form of a proof often conceals and does not reveal the thought processes and the mental efforts that went into its invention.

The two volumes are good for a self-study of the subject.

T. RAMESAN.

**Mechanisms of Inorganic Reactions.** By F. Basolo and R. G. Pearson. 2nd Edn. (Wiley Eastern Private Ltd., New Delhi), 1973. Pp. xi + 701. Price Rs. 40.00.

The publishers must be commended for bringing out a low-cost reprint of this well-known and authoritative book. An introductory chapter on fundamentals of coordination chemistry is followed by a detailed and critical account of the Theory of the Coordinate Bond. These two chapters might be read profitably by students of M.Sc chemistry courses of Indian Universities.

Two chapters are devoted to Substitution Reactions of Octahedral Complexes, one to kinetics and mechanisms and the other to stereo-chemical

changes. There follow chapters on Substitution Reactions of Square Planar Complexes and Oxidation-Reduction Reactions. It is pleasing to note that a chapter on Reaction of Transition Metal Organometallics has been added in the second edition reflecting the great activity in this field and the commercial importance of these reactions. The last chapter gives a concise account of Metal Ion Catalysis and Photochemistry.

The book is a thoroughly readable one. The treatment throughout is very incisive. The examples cited for illustrating specific points are carefully chosen. The welding of theoretical concepts and experimental results is no easy task particularly in this field and it can be said that the authors have succeeded admirably in this. The reviewer has no hesitation in recommending this book to all students of Inorganic Chemistry. For research workers in this area, it is a must.

S. S. KRISHNA MURTHY.

**Module VI—Basic Trigonometry.** By Leon J. Ablon. (Addison-Wesley Pub. Co., Reading, Massachusetts 01867, USA), 1974. Pp. 114.

In conformity with the title, the book aims at basic notions of angle and its measurement. The style of the book with neat figures may impress a reader without any mathematical background to take to trigonometry.

The book has been divided into eight lessons. Lessons 1 and 2 deal with angles by degrees and radians. Four lessons 3, 4, 5, 6 are set apart just for measurement of angles by ratios. Lesson 7 attempts an exposition to exhibit the properties of a triangle by figures. Lesson 8 is devoted to solve the right-angled triangle.

There are too many collections of problems and worked examples under each lesson, which are not necessary. The book is very elementary and may be useful for the beginner as an adjunct rather than a text book in trigonometry.

K. N. KAMALAMMA.

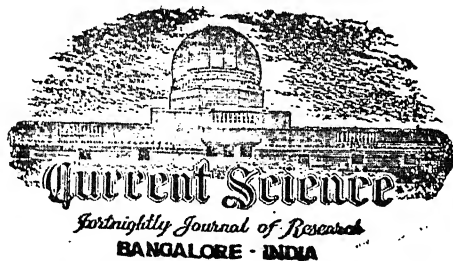
**Calculus with Probability for the Life and Management Sciences.** By Willard E. Baxter and Clifford W. Sloyer, (Addison-Wesley, Massachusetts, USA), 1973. Pp. xiii + 648.

This text book is written after considerable thought and experience. There is no point in merely saying that mathematics is important and

relevant to many fields of study. What is important is to be honest with the readers and the students for whom the mathematics text is meant by way of providing motivation through real-world problems. This is done by the authors in this book and they have clearly stated that they made no effort to be mathematically rigorous but that they are mathematically precise. At the same time the students from other sciences should have more than a nodding acquaintance with many mathematical concepts that arise in their sciences and for this they should undergo a formal training in mathematics under teachers from mathematics departments. The authors of this book are members of mathematics department and have rendered a good service by writing this book. They have consulted the books on biology, economics, etc., have had discussions with the professors in the life and management sciences, have got the students of these sciences involved by asking their reactions to the manuscript and all this have helped them in improving their treatment. For the present day students of life and management sciences, it is a must to study at least as much as is covered in this book as a first course. Some of them coming from mathematics or engineering background should take higher courses as well.

The book consists of 26 Chapters and various topics like sets, permutation-combinations, binomial theorem, probability on finite sample spaces, integral and differential calculus, continuous functions and limits, exponential and logarithm functions, linear differential equations, difference equations, functions of several variables, optimization are introduced. There is one chapter on mathematical models in the life and management sciences. The discussion of different topics in the book is not compartmentalized but the concepts are introduced and utilized when the need has arisen. This is clear from the Chapters on 'Probability and differential calculus', 'Improper integrals and normal density function' and 'Taylor polynomials and the Poisson processes'. Practically all the sections of every Chapter have problems for practice and answers to odd-numbered problems are provided. At the end of the book there is additional compressive problem set given Chapterwise. The level of the book is elementary and is meant for the beginners in life and management sciences with little background in training in mathematics.

V. G. THEKAR.



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ACID- AND BASE-CATALYSED TRANSFORMATIONS OF *m*-MENTHADIENES

BAKTHAN SINGARAM AND J. VERGHESE

Department of Chemistry, Christian Medical College, Vellore 632002

VERY little information<sup>1-3</sup> is available on the behaviour of *m*-menthadienes towards acid- and base-catalysts. For that reason we subjected *d*-*m*-mentha-1(6), 8-diene [*d*-sylvestrene] (I), *m*-mentha-1(6), 3(8)-diene [isosylvesterpine] (II) and *m*-mentha-1, 3(8)-diene [sylvesterpine] (III) to the action of trichloroacetic acid and potassium *t*-butoxide, catalysts already tested in the *p*-menthadiene field<sup>4-6</sup>.

Adopting essentially the procedure recommended for (+)-limonene<sup>4</sup>, the *m*-menthadienes were processed with trichloroacetic acid [Table I(A)].

isopropylidene derivatives, (III), is formed ~ 71 times that of (II).

Upon reacting isosylvesterpine with trichloroacetic acid, the dominant reaction is conjugation to (III) (71%); the isopropenyl derivatives (IV) (IV) and (V) amount to a total of only 13.5%. Clearly then, one of the routes of generating (III) from (I) is through (II).

On the other hand, the reactivity profile of sylvesterpine is in marked contrast to the above two *m*-menthadienes. Being a conjugated diene, it displays considerable stability; only 56% of the

TABLE I  
Transformations of *m*-menthadienes

Terpene* (Kind)	Catalysate			Analyses by g.l.c. (%)						
	Yield	$n_D^{20}$	$(\alpha)_D^{20}$	I <sup>a</sup>	II <sup>b</sup>	III <sup>c</sup>	IV	V	VI	VII <sup>d</sup>
(I)	2.3	1.5059	$\pm 0$	3.0	5.1	36.2	15.0	2.0	6.1	23.9
(II)	2.9	1.5047	$\pm 0$	3.4	5.0	71.0	4.8	5.3	0.5	10.0
(III)	1.9	1.5061	$\pm 0$	2.0	2.4	44.2	6.0	..	0.2	30.2

B										
Catalyst: Potassium <i>t</i> -butoxide† in DMSO‡; 85±2°; 3 hr										
(I)	3.7	1.4962	$\pm 24.7$	45.6	5.5	45.0	3.0	0.9	..	..
(II)	4.0	1.5051	$\pm 0$	12.2	8.2	73.0	4.3	2.3	..	..
(III)	3.9	1.5107	$\pm 0$	2.4	6.1	82.0	6.5	2.4	..	..

R <sub>f</sub>	0.73	0.88	0.86	0.76	0.30	0.93	0.90
----------------	------	------	------	------	------	------	------

\* 0.0031 mole; \*\* 0.031 mole; † 0.06 mole; ‡ 50 ml.

Tetrabromide, m.p. and mixed m.p.: (a) 137–138°; (b) 127–128°; (c) Maleic anhydride adduct m.p. and mixed m.p.: 306–308°; (d) n.m.r. (CCl<sub>4</sub>):  $\delta$  (7.0) (aromatic protons), further characterised by oxidation to isophthalic acid (dimethylester<sup>15</sup>, m.p. and mixed m.p. 64–65°).

Qualitatively the catalysates obtained are composed of (I), (II), (III), *m*-mentha-1, 8-diene [isylvestrene] (IV), *m*-mentha-1 (7), 8-diene (V), *m*-mentha-1-ene (VI) and *m*-cymene (VII). Nevertheless, the data throw light on the comparative stability and reactivity of these *m*-menthadienes.

Contrary to the belief that sylvestrene is stable<sup>7</sup>, only 3% of the hydrocarbon survived reorganisation. The proportion of isopropylidene to isopropenyl derivatives is in the approximate ratio 2:1 which reflects that in (I) the *exo* is more labile than the *endo* double bond. The shift of the nuclear double bond favours the liberation of (IV) (15%) rather than of (V) (2%)<sup>8</sup>. Of the

hydrocarbon suffered transformation. The endocyclic double bond wandered reluctantly within the ring to furnish (I) (2%) and (IV) (6%) but refused to migrate to the exocyclic position to afford (V).

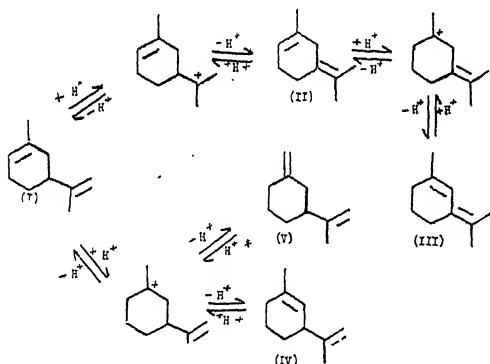
The isomerisations are accompanied by disproportionation to *m*-cymene (VII) and *m*-mentha-1-ene (VI). Sylvesterpine underwent maximum conversion to *m*-cymene (~30.2%). Since, in the catalysates, (III) is the richest species, it stands to reason that hydrogen transfer might have occurred mainly through this hydrocarbon, even though the participation of other *m*-menthadienes is not excluded.

For the base-catalysed reactions, the *m*-menthadienes were vigorously stirred and heated in an atmosphere of nitrogen with potassium *t*-butoxide in DMSO [Table I (B)].

Under the experimental conditions, the reactivity of these *m*-menthadienes is of the order (II) > (I) > (III). Only 18% of (III) has been changed to other isomers, again substantiating its great stability. Sylveterpinolene is the major product of isomerisation of (I) and (II), the yield being respectively 45% and 73%. This is in accord with the fact that base-catalysed isomerisations stimulate conjugation<sup>9</sup>. As in acid-catalysed isomerisations, the data suggest the reaction sequence: (I) → (II) → (III). Only superficial conversion of these *m*-menthadienes to sister isomers (IV) and (V) has taken place. The most distinguishing feature of the base-catalysed reactions is that the isomerisates are remarkably free from products of disproportionation<sup>10</sup>.

In general, the present investigation has clearly established the interconvertibility of (I), (II) and (III).

The acid-catalysed transformations can be adequately explained by assuming the intermediate formation of carbonium ions<sup>11</sup>. Thus, the steps leading to the four isomers (II)–(V) from *d*-sylvestrene (I) can be pictured as in Scheme I.



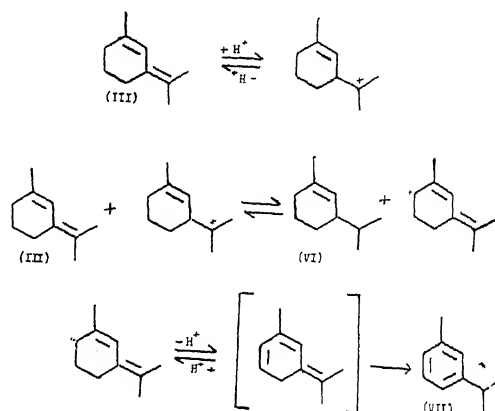
SCHEME I. Acid-catalysed rearrangement of *d*-sylvestrene.

*m*-Cymene (VII) and *m*-menth-1-ene (VI) may be generated through  $H^+$  induced disproportionation of *m*-menthadienes. We advance tentative Scheme II, with sylveterpinolene (III) as an example.

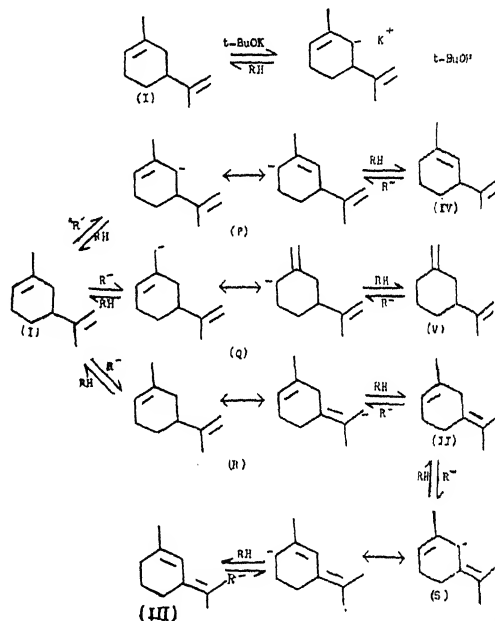
A mechanism involving allylic carbanion<sup>12</sup> may be invoked to accommodate the base-catalysed rearrangements of *m*-menthadienes as illustrated for *d*-sylvestrene (I) in Scheme III.

The allylic resonance hybrids (P), (Q), (R) and the bialylic carbanion (S) are the probable pre-

cursors of the isomers (IV), (V), (II) and (III) respectively.



SCHEME II. Disproportionation of sylveterpinolene.



SCHEME III. Base-catalysed rearrangement of *d*-sylvestrene.

\* $R^-$  represents a *m*-menthadienyl ( $C_{10}H_{15}$ )<sup>-</sup> anion,  $K^+$  is omitted.

#### EXPERIMENTAL

For general experimental details see Part XXXVII<sup>13</sup>.

**Materials.**—Hydrocarbons (I), (II) and (III) were prepared by routes previously described<sup>1,2</sup>.

**Procedure.**—The *m*-menthadienes were reacted with trichloroacetic acid and potassium *t*-butoxide as for (+)-limonene<sup>1</sup> and car-3-ene<sup>14</sup> respectively.

The catalysates were analysed by t.l.c., g.l.c., n.m.r. and chemical methods (Table I).

#### ACKNOWLEDGEMENT

One of us (B. S.) is grateful for support by the C.S.I.R. (New Delhi).

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### EFFECT OF CROP SEQUENCE ON *ASPERGILLUS FLAVUS* INFESTATION AND AFLATOXIN ACCUMULATION IN GROUNDNUT (*ARACHIS HYPOGAEA* L.)

P. SUBRAHMANYAM AND A. S. RAO\*

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**A** *SPERGILLUS FLAVUS* is an ubiquitous fungus known to invade a variety of agricultural and food commodities and produce a group of toxic metabolites known as aflatoxins, which are highly carcinogenic. Only a few strains are toxin producing and groundnut is said to be one of the best substrates for aflatoxin production<sup>14</sup>. This fungus may invade groundnut pods while developing in the soil or after the harvest and subsequent storage<sup>1-7,8</sup>.

Crop rotation is said to be one of the most beneficial agronomic practices to control certain diseases and also to regain soil fertility. Pettit and Taber (1968) found that groundnut harvested from lands planted with the same crop during the previous season also was more highly infested with fungi and contained more aflatoxins than groundnut raised on lands planted with rye, oats, melons or potatoes as the previous crop. Joffe and Lisker (1970) also found high fungal infestation to groundnut kernels in fields previously sown with groundnut than non-groundnut soils.

The objective of present investigation is to examine the effect of crop sequence on *A. flavus* and other fungal populations in soil, rhizosphere and geocarposphere, shells and kernels at various stages of crop development and also aflatoxin content at harvesting period.

Field plots (approx. 7 × 12 m in size) with different crop sequence history were selected near

S.V. University Campus, Tirupati (A.P.). The plots had the following crop sequence in *Kharif* and *Rabi* since three years. Plots with vegetable crop (brinjal, chilli, tomato and mesta as mixed crops) in *Kharif* and groundnut in *Rabi*; rice in *Kharif* and groundnut in *Rabi* and groundnut in both seasons. In each case three replicated plots were maintained and cultivated under similar agronomic practices. Fungal populations, pod infestation and aflatoxin accumulation were studied in late *Rabi* season (February–May) of 1972 and 1973. The soils are of red sandy type, low in organic matter content and colloidal content<sup>12</sup>.

For the estimation of fungal populations, ten plants were pulled up from different regions of each replicated plot. The plants and also the soil in between the rows (control soil) were brought to the laboratory in polythene bags. The rhizosphere and geocarposphere mycofloras were estimated according to the method of Rao (1962) and Joffe (1969) respectively. Shell and kernel infestation was examined by the method of McDonald (1970). At the time of harvest about 15,000 pods from each replicated plot were collected and graded into (1) "Undamaged pods" (without any kind of damage) and (2) "damaged pods" (rotted, insect-bored, injured, etc.). Aflatoxins were estimated in both undamaged pods and damaged pods by the method of Pons et al. (1966) and confirmed by bioassay using *Bacillus mageterium* (NRRL 1368) and *B. brevis* (NRRL 1874) following the method of Burmeister and Hesseltine (1966).

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There was a positive rhizosphere and geocarposphere response on fungal populations as reported by the previous workers<sup>4,5,11</sup>. This is mostly due to exudation of roots and pods<sup>12,13</sup> and also due to sloughed-off root and shell tissue at maturing stage. In both years high *A. flavus* population was seen in soil rhizosphere and geocarposphere in plots with groundnut as previous crop (Table I).

This may be due to left-over pods and other plant waste like senesced leaves of groundnut in soil on which *A. flavus* may colonize and survive well than on paddy and vegetable waste. High *A. flavus* infestation to shells and kernels and also high aflatoxin accumulation was noticed in both undamaged and damaged pods in plots previously sown with groundnut. In all cases aflatoxin accumulation is

TABLE I

*Aspergillus flavus* and other fungal populations in soil (S), rhizosphere (R) and geocarposphere (G) of groundnut (Thousands/gram soil)

Year	Previous crops in the field		<i>A. flavus</i>				Other fungi			
			20*	45	75	105	20	45	75	105
1972	Vegetables	S	0	2.2±	4.1±	5.5±	5.1±	9.5±	8.5±	15.1±
				0.46	0.75	0.80	0.82	0.64	0.56	1.28
		R	1.2±	0	12.1±	10.2±	12.0±	74.1±	60.4±	64.0±
				0.21	0.82	0.68	1.96	10.42	8.96	9.52
		G	..	..	4.2±	10.0±	..	..	32.5±	28.2±
				..	0.71	0.71	..	..	6.21	5.10
	Rice	S	0	0	2.1±	2.0±	3.0±	12.0±	4.2±	10.2±
				0	0.42	0.42	0.22	2.10	0.36	0.68
		R	0	8.5±	7.2±	8.0±	16.2±	76.2±	45.5±	52.1±
				0.61	0.58	0.75	1.42	10.10	6.10	10.0
		G	..	..	5.2±	6.1±	..	..	22.3±	25.5±
				..	0.35	0.40	..	..	4.54	4.0
	Groundnut	S	12.1±	8.9±	16.0±	18.0±	8.0±	15.2±	12.0±	14.2±
				0.92	0.71	1.21	0.58	0.98	1.98	2.10
		R	16.1±	34.0±	12.0±	15.0±	24.8±	64.5±	68.0±	70.1±
				2.00	2.98	1.50	3.92	8.86	8.58	9.28
		G	..	..	16.6±	18.8±	..	..	45.4±	40.0±
				..	2.80	2.22	..	..	7.0	3.82
1973	Vegetables	S	1.2±	0	2.1±	5.5±	3.1±	7.8±	3.0±	15.2±
				0.1	0.21	0.65	0.52	0.92	0.6	2.0
		R	5.1±	12.1±	0	15.1±	15.2±	78.2±	52.2±	60.0±
				0.42	1.75	1.56	2.1	6.88	5.42	5.58
		G	..	..	5.1±	12.1±	..	..	40.0±	25.3±
				..	0.64	1.82	..	..	5.00	2.92
	Rice	S	5.3±	0	0	4.0±	3.2±	9.2±	4.2±	10.2±
				0.30	0	0.50	0.42	1.10	0.52	1.68
		R	3.0±	0	5.0±	10.0±	10.5±	69.0±	35.1±	53.6±
				0.42	0.62	2.22	1.50	16.48	4.82	15.10
		G	..	..	3.2±	7.0±	..	..	34.5±	38.0±
				..	0.56	2.0	..	..	4.0	4.0
	Groundnut	S	3.1±	2.2±	12.5±	8.8±	5.1±	11.2±	14.4±	18.8±
				0.50	0.32	1.92	0.66	1.0	1.82	2.0
		R	4.5±	14.8±	27.0±	18.2±	18.6±	92.5±	54.2±	60.1±
				0.62	2.5	5.0	2.92	12.52	5.42	6.42
		G	..	..	18.8±	25.5±	..	..	45.2±	53.2±
				..	2.86	4.46	..	..	5.82	4.98

\* Number of days after planting.

TABLE II

Isolation frequency of *A. flavus* and other fungi from groundnut shells and kernels and aflatoxin content in kernels at the time of harvest

Year	Previous crop in the field	Shells						Kernels						Pod condition		Aflatoxin content <sup>b</sup> (µg/kg)	
		<i>A. flavus</i>			Other fungi			<i>A. flavus</i>			Other fungi			% un-damaged pods	% damaged pods	un-damaged pods	damaged pods
		60 <sup>a</sup>	85	105	60	85	105	60	85	105	60	85	105				
1972	Vegetables	1 <sup>a</sup>	10	25	12	34	38	0	0	16	0	15	45	92.1	7.9	Tr <sup>c</sup>	2761± 455.42
	Rice	0	0	20	6	52	25	0	5	5	0	12	31	87.0	13.0	Tr	2676± 450.56
	Groundnut	5	12	52	10	45	92	0	5	53	5	48	48	90.8	9.2	140± 18.5	3736± 586.38
1973	Vegetables	2	20	54	15	38	68	0	2	35	0	5	65	93.0	7.0	Tr	3608± 320.42
	Rice	0	25	30	10	22	53	0	0	17	12	18	24	84.5	15.5	0	3696± 758.82
	Groundnut	0	32	65	18	54	60	0	15	52	15	24	42	91.4	8.6	854± 105.50	6180± 1028.98

<sup>a</sup>=Number of days after planting. <sup>b</sup>=Average of five replications. <sup>c</sup>=Traces (less than 1 µg.).<sup>a</sup>=Values expressed as percentage of shells or kernels from which *A. flavus* or other fungi grew out onto agar.

more in damaged pods, than in undamaged pods. The kernel surface of the damaged pods will be exposed to the surrounding soil and becomes more susceptible for fungal invasion. But in undamaged pods the intact shell and seed coat act as a natural barrier for fungal invasion.

The results relating to the counts of *A. flavus* (actual values as percentage on totals) on shells and kernels at 105 days have been subjected to statistical analysis according to the analysis of variance method on factorial basis. The mean actual values of *A. flavus* counts at 105 days for the three main factors: (1) 1972 vs. 1973 (years), (2) shells vs. kernels (pod parts) and (3) Vegetables vs. rice vs. groundnut (previous crop) have become statistically significant even at 1% probability. Considering the percentage values, it is seen that the effects of the main factors have not become statistically significant even at 5% probability. However, it is noted that the effects of the previous crops are significant at 10% probability.

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# KINETIC COMPLEMENT FIXATION TEST FOR RAPID IDENTIFICATION OF JAPANESE ENCEPHALITIS AND WEST NILE VIRUSES

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## ABSTRACT

A kinetic CF test was employed for rapid identification of the viruses of JE-WN complex. Addition of haemolytic system after incubation of antigen, antibody and complement for 2 hours at 4° C gave reactions which could differentiate the two viruses with ease. Two strains of JE and one strain of WN virus were identified in this manner. Identification of the isolates were confirmed by employing absorbed mono-specific immune sera.

**A** RAPID identification of Japanese encephalitis (JE) and West Nile (WN) viruses is often difficult due to the close antigenic relationship between the two viruses. This difficulty was overcome by Pavri and Shaikh<sup>1</sup> by employing mono-specific JE/WN absorbed sera in routine complement fixation tests. A 20% infected mouse brain suspension in normal saline could be used as antigen for rapid identification. Non-availability of monospecific absorbed sera pose problems, as most laboratories are not equipped with a high-speed centrifuge. By changing certain parameters, such as the reaction time, it was possible to establish the identity of the new isolates. The present communication deals with the procedures followed for their identification.

P 20778 strain of JE virus and G 22886 strain of WN virus were employed in the complement fixation tests. Sucrose acetone extracted mouse brain antigens were prepared according to the method described by Clarke and Casals<sup>2</sup>. For rapid identification, 10–20% infected mouse brain suspensions in normal saline were centrifuged at 2500 g for one hour and the supernatants were employed as antigens. Virus strains 724038 (JE) and 724268 (WN) were isolated from mosquitoes collected in Andhra Pradesh (Rodrigues: personal communication) and strain 733690 (JE) was isolated from human brain during JE epidemic in West Bengal (Sarkar)<sup>3</sup>. Normal mouse brain antigen was prepared in a similar manner.

Hyperimmune sera/ascitic fluids were raised in mice by inoculating intraperitoneally five doses of live virus. The mice were bled 4–7 days after the last dose. Immune ascitic fluids were collected from hyperimmunized mice inoculated with Erlich ascitic tumour cells. These cells were maintained in this laboratory after receipt from Dr. J. K. Sarkar of Calcutta School of Tropical Medicine and Hygiene. JE/WN monospecific absorbed sera were prepared according to the method described by Pavri and Shaikh<sup>1</sup>.

Complement fixation (CF) tests were performed by the method described by Pavri *et al.*<sup>4</sup>. A kinetic CF test was carried out by varying the incubation period. The extracted antigens or quick antigens, the hyperimmune sera/ascitic fluids, and 2–2.5 units of complement were incubated at 4° C. Sensitized sheep erythrocytes were added at intervals of 0 hour, 1 hour, 2 hours and overnight incubation of the test at 4° C. The plates were then incubated at 37° C and the test was read one hour after the incubation.

The extracted antigens reacted rapidly with the hyperimmune sera against the homologous antigen. There was either no reaction or a low reaction with heterologous sera when the test was incubated for 0 hour to 2 hours. The homologous as well as heterologous titres increased with the increase in incubation period.

Another parameter, the concentration of antigen, also had an effect on the detection of homologous as well as heterologous antibodies in relation to the period of incubation (Table I). A unit of antigen is defined as the highest dilution of the antigen which fixes 2.5 units of complement in the presence of homologous mouse immune serum. Sixteen units of antigens gave more cross reactions with heterologous sera in overnight incubation of the test system and as the concentration of antigens decreased the reactions with heterologous sera were less marked especially when 1–2 units of antigens were employed. The titres of homologous sera were not affected with the decrease in the concentration of antigens in overnight incubation. The reaction was demonstrated in one hour when more than four units of antigens were employed.

The kinetic CF test with the saline extracted mouse brain antigens of 724038 and 733690 strains gave a sharp difference in their reactions with JE hyperimmune sera when 2 hour reaction time was allowed (Table II). Identification of both the strains of JE virus were further confirmed by demonstrating a positive reaction with monospecific

TABLE I  
*Kinetic CF test effect produced by variable quantity of antigen*

Antigen	No. of units of antigen	HOURS*							
		0		1		2		18	
		JE imm.	WN imm.	JE imm.	WN imm.	JE imm.	WN imm.	JE imm.	WN imm.
JE	32	32	<8	256	16	256	32	512	128
	8	8	<8	256	<8	256	32	512	128
	4	<8	<8	256	<8	256	16	512	128
	2	<8	<8	16	<8	32	<8	UNS	16
	1	<8	<8	<8	<8	<8	<8	512	<8
	<1	<8	<8	<8	<8	<8	<8	UNS	<8
WN	16	8	32	32	128	32	128	256	512
	8	<8	16	32	128	32	128	128	512
	4	<8	<8	16	128	16	128	64	512
	2	<8	<8	<8	16	<8	128	16	512
	1	<8	<8	<8	<8	<8	16	<8	512
	<1	<8	<8	<8	<8	<8	<8	<8	<8
Normal	..	<8	<8	<8	<8	<8	<8	<8	<8

Imm. = Immune sera, UNS = Unsatisfactory, \* Different time of incubation at 4° C before addition of sensitized erythrocytes. Normal control sera in all cases yielded titres of <8.

TABLE II  
*Kinetic complement fixation test identification of new isolates*

Immune sera	Antigens											
	724038			724268			733690			Homologous		
	*0	2	18	0	2	18	0	2	18	0	2	18
JE imm.	16	128	≥256	<8	32	64	32	≥128	≥128	8	64	≥256
WN imm.	<8	32	128	256	≥512	≥512	<8	<8	32†	256	≥512	≥512
										†16	16	64
JE imm. (absorbed Sr.)	8	64	128	—	—	8	—	—	128	8	64	128
WN imm. (absorbed Sr.)	<8	<8	<8	—	—	64	—	—	<8	<8	16	≥258
N Sr./PF	<8	<8	<8	<8	<8	<8	<8	<8	<8	—	—	—
Identification of the isolate	JE			WN			JE					

\* Hours of incubation at 4° C before addition of sensitized erythrocytes, † Homologous titres of antiserum employed in that particular test. — = Not done. Normal control antigen did not react with the immune sera.

absorbed JE immune serum as well as in neutralization test. The isolate 724268 gave a specific reaction with West Nile immune serum when the sensitized cells were added immediately. Further confirmation was obtained by demonstrating reaction with WN specific absorbed sera.

Hatgi and Sweet<sup>5</sup> found that by varying a number of parameters of the test such as antigen concentration and the reaction period they could profitably apply the test to type dengue viruses and in some cases also establish intratypic strain variations. The kinetic CF test was more specific at lower antigen

concentrations. Cross reactions were more marked when excess antigen was employed. In the present studies the titres of the crude antigens were not determined. The checkerboard titrations of JE-WN antigens against homologous as well as heterologous hyperimmune sera indicated that the reactions with heterologous sera diminished proportionately with the reduction in the concentration of antigens, however, reactions with the homologous sera were not affected to the same extent.

The sharp differentiation in reactions of the hyperimmune sera of JE-WN complex in less than



2 hours has made this test a useful tool especially when monospecific immune sera not available.

#### ACKNOWLEDGEMENT

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## DISCONTINUITY IN THE LARVAL DISTRIBUTION OF PHORONIDA AND BRACHIOPODA IN THE INDIAN OCEAN

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#### ABSTRACT

Distribution of Phoronid and Brachiopod larvae in Indian Ocean was studied on the basis of the zooplankton samples collected during the International Indian Ocean Expedition. The striking feature is the discontinuous distribution of these larvae. The larvae of Phoronida prefer relatively low salinity waters while the Brachiopod larvae have high tolerance to changes in salinity. The abundance of both Brachiopod larvae and Actinotrocha in the Bay of Bengal suggests the richness of the adults in this region of the Indian Ocean.

**P**HORONIDA and Brachiopoda have free swimming larval stages in their life-histories and these larvae are familiar constituents of plankton. Both Phoronida and Brachiopoda lead a benthonic existence. Phoronids, so far recorded, are limited to the shallow waters of tropical and temperate zones<sup>1</sup>. Brachiopods are exclusively marine forms and occur in all seas from the intertidal zone to depths of 500 m<sup>1</sup>. Their meroplanktonic larvae serve as links and maintain genetic continuity between populations spatially isolated from one another<sup>2</sup>.

There are only a few reports on these larvae from the Indian Ocean and adjacent seas. The earlier records of the Brachiopod larvae are the occurrence of *Lingula* larvae in the Gulf of Aden, South of Red Sea, off the Mysore coast, west coast of Sumatra and of *Pelagodiscus* larvae from the southwestern part of India<sup>3-5</sup>. A number of Actinotrocha are known of which the adult has not been identified<sup>1</sup>.

During the International Indian Ocean Expedition from 1960 to 1965, zooplankton samples amounting to 1927 were collected from the Indian Ocean between the Lat. 25° N to 46° S and Long. 20° to 120° E. Most of the samples were taken with an Indian Ocean Standard Net from a depth

of 200 m to the surface or in the continental shelf from the bottom to the surface<sup>6</sup>. The data obtained from these zooplankton samples form the basis of the present study.

#### ACTINOTROCHA

The fully developed larva has an elongated body varying from less than 1 to 5 mm in length<sup>1</sup>. The planktonic existence of the larva extends probably to several weeks. Actinotrocha are represented in 4.1% of the samples. Maximum incidence of the larvae was found to be in the Bay of Bengal, off the coast of Somalia and off the southeast coast of Africa (Fig. 1). A striking feature is the discontinuous distribution of the larvae as they were absent or sparsely represented in the Arabian Sea, Central Indian Ocean and eastern part of the Indian Ocean between the Equator and Lat. 30° S and Long. 68° to 120° E. Their seasonal occurrence and other details are given in Table I. The hydrographical data at the stations, from which high abundance of the larvae was recorded, have a temperature range 13.69°-26.48° C, salinity 32.86-35.46‰, oxygen 0.36-5.40 ml/l and phosphate phosphorus 0.16-1.33 µg at/l. With the exception of three records, they were never found at stations where maximum

salinity was 36‰ or near this value. It is probable that the larvae prefer relatively low salinity waters.

common in the Bay of Bengal, Gulf of Aden and off the southwest coast of India (Fig. 2). These larvae also exhibit a discontinuous distribution,

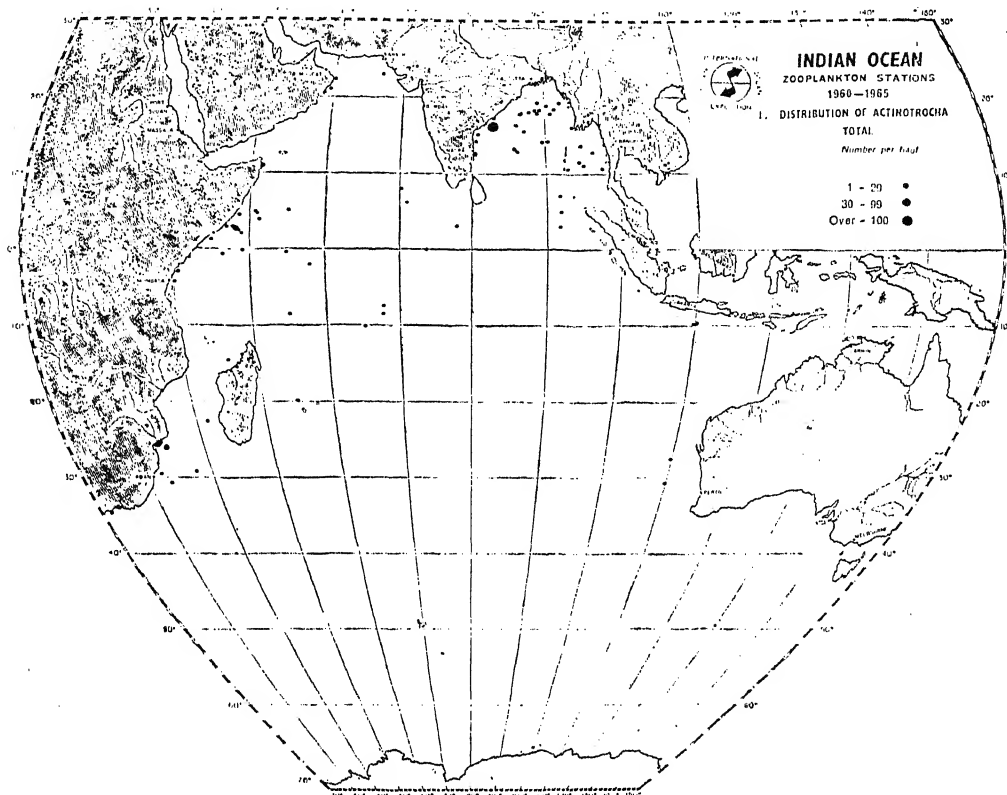


Fig. 1. Population densities of *Actinotrocha* in the Indian Ocean.

TABLE I

*Distribution of Actinotrocha and Brachiopod larvae in the Indian Ocean*

Larvae	Total No.	April 16–October 15 (Southwest monsoon period)	October 16–April 15 (Northeast monsoon period)	Day Average No./haul	Night Average No./haul	Maximum abundance	
		Average No./haul	Average No./haul			Area and density	Month
<i>Actinotrocha</i>	981	10.6	14.0	8.1	23.0	287 (off the Andhra Coast)	January
Brachiopod larvae	292	3.6	7.2	5.6	5.6	43 (Gulf of Aden)	December

#### BRACHIOPOD LARVAE

The fully developed larva has a bivalved shell, with a diameter ranging from 0.3–1.5 mm<sup>1</sup>. These larvae were found in 2.7% of the zooplankton samples. Brachiopod larvae were more

being absent in the northern Arabian Sea and in the Central Indian Ocean. Table I shows important features in their distribution. The maximum number of larvae were found at a station located near the mouth of the Red Sea where hydrographical

conditions for the upper 200 m were: temperature  $22.79^{\circ}$ – $27.10^{\circ}$  C, salinity  $36.55$ – $39.59$ ‰, oxygen content  $1.86$ – $4.55$  ml/l and phosphate phosphorus  $0.27$ – $6.89$   $\mu$ g at/l. Contrary to the comments made by Hyman<sup>1</sup> that they have a preference to cooler waters, that present data show that the larvae prefer tropical and northern subtropical areas of the Indian Ocean, their southern

wind and currents<sup>3</sup>. The abundance of both Brachiopod larvae and Actinotrocha in the Bay of Bengal indicates the richness of adults in this region of the Indian Ocean. Intensive surveys of both adults and larvae of Brachiopods and Phoronids in the Bay of Bengal may reveal the ecological factors which govern the distribution of these larvae and adults in this region.

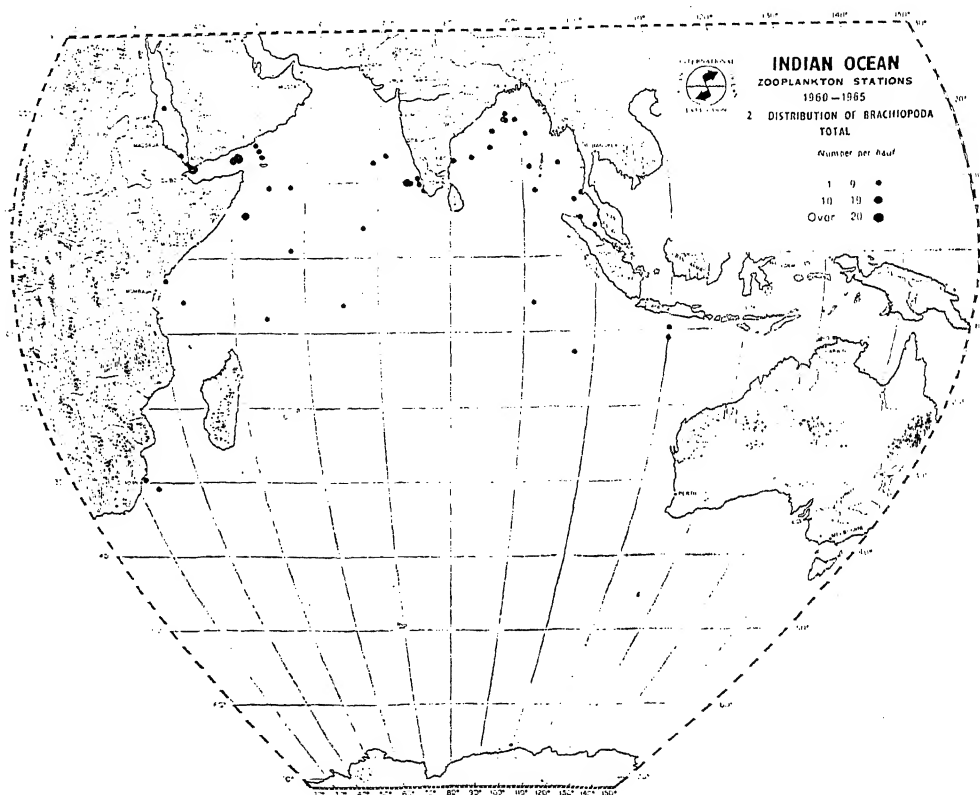


FIG. 2. Population densities of Brachiopod larvae in the Indian Ocean.

boundary being Lat.  $30^{\circ}$  S. Occurrence of Brachiopod larvae in the high saline waters of the Red Sea as well as in low salinity waters of the Bay of Bengal indicates their high tolerance to changes in salinity. Muir-Wood<sup>3</sup> has also recorded a discontinuous distribution in the three genera of adult Brachiopods of the Indian Ocean.

Brachiopods tend to live in congregation and the larvae settle near the adults in favourable areas. The power of dispersal of these larvae seem to be limited even when their movement is assisted by

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## LETTERS TO THE EDITOR

### ELECTRICAL PROPERTIES OF A NEW TYPE OF COMPOUNDS $A^{3+}A^{2+}B^{1+}O_4^{2-}$

THE structural characteristics of a new type of compounds with the general formula  $A^{3+}A^{2+}B^{1+}O_4^{2-}$ , where  $A^{3+} =$  a rare earth cation,  $A^{2+} = Li^+, Na^+, K^+$  and  $B^{1+} = Ti^{4+}, Hf^{4+}, Zr^{4+}$  have recently been reported<sup>1</sup>. This note deals with their electrical properties (dielectric constant and loss tangent).

The methods of preparation of the pellets and their dielectric measurements are as described earlier<sup>2,3</sup>. The results are shown in Table I for some of the representative samples in the series.

TABLE I

Dielectric constant ( $\epsilon$ ) and loss tangents ( $\tan \delta$ ) of  $A^{3+}A^{2+}B^{1+}O_4^{2-}$  compounds at 26° C, 10 kHz and 50 V/cm across the pellets

Compound	Structure type*	$\epsilon$	$\tan \delta \times 10^2$
NdLiHfO <sub>4</sub>	.. S	22	3.0
SmNaTiO <sub>4</sub>	.. S	32	2.5
SmKHfO <sub>4</sub>	.. S	26	3.2
GdNaTiO <sub>4</sub>	.. S	28	2.1
GdKZrO <sub>4</sub>	.. S	30	2.9
DyNaTiO <sub>4</sub>	.. S	26	1.8
DyKZrO <sub>4</sub>	.. S	27	2.2
YKTiO <sub>4</sub>	.. F	32	3.2
LaNaTiO <sub>4</sub>	.. F	38	3.0
NdLiTiO <sub>4</sub>	.. F	30	2.8

\* S = Scheelite ( $CaWO_4$ ) type, F = Fergussonite ( $YTaO_4$ ) type.

It is seen that the dielectric constant and  $\tan \delta$  values go on decreasing with the decreasing size of the rare earth cation, provided the  $A^{2+}$  and  $B^{1+}$  ions are kept constant. It is known<sup>4</sup> that the covalent character in the lanthanides increases with the decreasing ionic size. The dielectric constant and the loss tangent therefore seem to be directly proportional to the degree of covalency of the Ln-O bond in these compounds. Similar observations have been recorded before in this laboratory<sup>5</sup>. Our values of the dielectric constants are comparable to those reported<sup>6</sup> for  $PbWO_4$  (23.6 || to a axis and 31.0 || to c axis) and higher than  $CaWO_4$  (11.7 || to a axis and 9.5 || to c axis). In absence of a single crystal data on these compositions, however, it is not possible to compare the anisotropy of the dielectric constants in these compounds with those reported for  $PbWO_4$  and  $CaWO_4$ , since polycrystalline structures yield

an average of the values in different directions. No dispersion of the dielectric constant was observed upto 500 kHz. These results are in conformity with those of Brower and Fang<sup>7</sup> on scheelites. The electrical resistivity of these compositions are reported elsewhere<sup>8</sup>.

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### BOND AND MOLECULAR POLARIZABILITIES OF BENZOPHENONE

In earlier communications<sup>1-3</sup>, we have reported molecular polarizabilities of some inorganic molecules<sup>1</sup>, organic molecules with ring and chain structure<sup>2</sup> and carbonyls<sup>3</sup>, using a semi-empirical quantum mechanical  $\delta$ -function model<sup>4</sup> of chemical bonding. We extended the use of this model to comparatively larger organic molecules with single benzene ring, two and three benzene rings fused at a time. Even though  $\delta$ -function model is basically one-dimensional in nature, the values calculated are in good agreement with the experimental values of polarizability. In this communication, another interesting molecule has been examined where the two benzene rings are connected through a carbonyl group (Benzophenone). Retaining the concept of additivity principle of bond polarizability, the parallel and perpendicular components of bond polarizability ( $\alpha_{||}$ ) and ( $\alpha_{\perp}$ ), average polarizability ( $\bar{\alpha}_m$ ), and contribution by non-bonding electrons ( $f_j, a_j$ ) have been computed for the molecules in question and are presented in Table I. The

TABLE I  
Bond and molecular polarizabilities (in  $10^{-25} \text{ cm}^3$ ) of benzophenone  $\text{C}_{12}\text{H}_{10}\text{O}$

Bond	Bond length (in Å)	$\alpha_{ijb}$	$\Sigma \alpha_{ijp}$	$\Sigma f_j \alpha_j$	$\Sigma 2\alpha_L$	Total polarization contribution
$\text{C}=\text{O}$	1.25	19.79	15.39	3.260	314.37	$\Sigma \alpha_{ijp} = 319.99$ $\Sigma f_j \alpha_j = 3.26$
$(\text{C}-\text{C})_g$	1.50	21.12	42.25	..	..	$\Sigma 2\alpha_L = 314.37$
$(\text{C}-\text{C})_x$	1.40	16.13	193.61	..	..	$\bar{\alpha}_{\text{M}} = 212.54 \text{ (cal.)}$ $\bar{\alpha}_{\text{M}} = 212.00 \text{ (obs.)}$
$(\text{C}-\text{H})$	1.08	7.15	68.74	..	..	(Ref. 6)

$\sigma_{ijp} = \alpha_{ijb} \sigma$ ,  $\sigma = \exp. - (X_1 - X_2)^2 / 4$  a polarity correction term in the case of hetero-diatomics and  $X_1$  and  $X_2$  are the electronegativities of the constituent atom.

various internuclear separations have been taken from the compilation by Sutton<sup>5</sup>. The atomic polarizabilities (in  $10^{-25} \text{ cm}^3$ ) used for C, H and O are 9.92, 5.92 and 5.92 respectively. The  $\delta$ -function strength (atomic units) are 0.846, 1 and 1. The components of molecular polarizabilities of benzophenone  $\alpha_{aa'}$ ,  $\alpha_{bb}$  and  $\alpha_{cc}$  have been recently reported<sup>6</sup> to be  $22.22 \text{ Å}^3$ ,  $23.03 \text{ Å}^3$  and  $18.62 \text{ Å}^3$ . The average molecular polarizability of this molecule thus obtained  $212 \times 10^{-25} \text{ cm}^3$  is in excellent agreement with the calculated value of  $212.54 \times 10^{-25} \text{ cm}^3$  (see Table I).

Suffixes  $g$  and  $x$  as evident from Fig. 1.

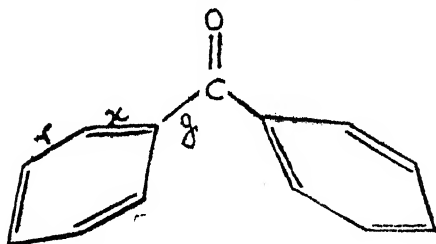


FIG. 1. Benzophenone.

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### PREPARATION OF RED MERCURIC SULPHIDE ( $\alpha$ )

THE red mercuric sulphide can be prepared, according to Venkataramaiah and Rao<sup>1</sup>, either by decomposing  $\text{Hg}(\text{SH})\text{CNS}$  at  $130\text{--}140^\circ \text{C}$  in air or by passing hydrogen sulphide into a warm mercuric solution in presence of acetic acid and excess of ammonium thiocyanate or thiourea. Audieth *et al.*<sup>2</sup> obtained the same compound by passing hydrogen sulphide into a hot solution of mercuric acetate and ammonium thiocyanate in presence of acetic acid. In this article a method for the preparation of red mercuric sulphide by passing hydrogen sulphide through a solution of mercuric chloride, ammonium thiocyanate and thiourea at room temperature is described.

**Experimental.**—Hydrogen sulphide was passed, at room temperature, into a solution of 0.1 M mercuric chloride, 0.5 M ammonium thiocyanate and 0.5 M thiourea with occasional stirring. The black precipitate obtained at the initial stages turned reddish brown slowly. Hydrogen sulphide was passed till the entire precipitate turned reddish brown. This precipitate turned red when left overnight with mother liquor. The red solid was washed with water and dried at  $100\text{--}105^\circ \text{C}$ . The density was found to be  $8.03 \text{ g cc}^{-1}$  at  $30^\circ \text{C}$  (lit. value<sup>3</sup>  $8.10 \text{ g cc}^{-1}$ ).

When mercuric chloride, ammonium thiocyanate and thiourea are mixed in the ratio 1:1:1 then reddish brown precipitate can be obtained within 10–12 minutes. If the ratio is 3:2:1 then hydrogen sulphide is to be passed for 20–25 minutes. In both cases the sulphide was exposed to atmosphere, in shade, for 2–3 months and no change in colour was noticed.

Further, it was found that the filtrate of the experiment could be utilized for the preparation of

further quantities of red sulphide. Thus the new method avoids the use of acetic acid and elevated temperatures while the filtrate containing thiourea and ammonium thiocyanate could be utilised in subsequent experiments.

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### A ROUGH ESTIMATION OF THE SOLUBILITY PARAMETERS OF STYRENE-ACRYLONITRILE COPOLYMERS

THE process of dissolution of a polymer can be related to the heat of dilution parameter  $K_1$  and to the solubility parameter of the solvent  $\delta_1$  and of the polymer  $\delta_2$ , by the equation<sup>1-3</sup>.

$$\kappa_1/V_1 = (\delta_1 - \delta_2)^2/RT \quad (1)$$

where  $V_1$  is the volume fraction of the solvent. Thus when the difference between the solubility parameters of the two components is small, the dissolution is higher as the solvent is a good one. Since<sup>3</sup>

$$\psi_1 - \kappa_1 = 1/2 - \chi_1 \quad (2)$$

where  $\psi_1$  is the entropy parameter and  $\chi_1$  is the enthalpy parameter, a rearrangement of eq.(1) using eq. (2) leads to the equation

$$\frac{V_1(\delta_1 - \delta_2)^2}{RT} = \chi_1 + \chi_s \quad [\chi_s = \psi_1 - 1/2]$$

and

$$\left\{ \frac{\delta_1^2}{RT} - \frac{\chi_1}{V_1} \right\} = \frac{2\delta_1\delta_2}{RT} - \left\{ \frac{\delta_2^2}{RT} - \frac{\chi_s}{V_1} \right\} \quad (3)$$

A plot of  $[\delta_1^2/RT - \chi_1/V_1]$  vs  $\delta_1$  gives a straight line from the slope of which  $\delta_2$  can be evaluated, if  $\chi_s$  is either negligibly small or constant for a set of solvents.

The values of  $\delta_2$  are evaluated for styrene-acrylonitrile copolymers. The estimation of  $\delta_2$  by this method, though not absolute, gives an idea about the nature of the solvent.

**Experimental.**—Three samples of styrene-acrylonitrile (St-AN) random copolymers of different composition, were prepared by the free radical polymerisation method, using benzoyl peroxide as the initiator. The compositions of these samples [0.274(SA<sub>1</sub>), 0.385(SA<sub>2</sub>) and 0.475(SA<sub>3</sub>) acrylo-

nitrile content] were estimated by nitrogen analysis and I.R. spectra. Fractionation was carried out in CHCl<sub>3</sub>-methanol system at 30°C. Molecular weights were determined<sup>5</sup> by light scattering technique at 5461 Å. The limiting viscosity numbers were evaluated<sup>6</sup> in dimethylformamide (DMF),  $\gamma$ -butyrolactone ( $\gamma$ -BL) and ethylacetate (EAc) at 30°C. From the Stockmayer-Fixman (S-F) plot (Fig. 1) the long range interaction parameter B

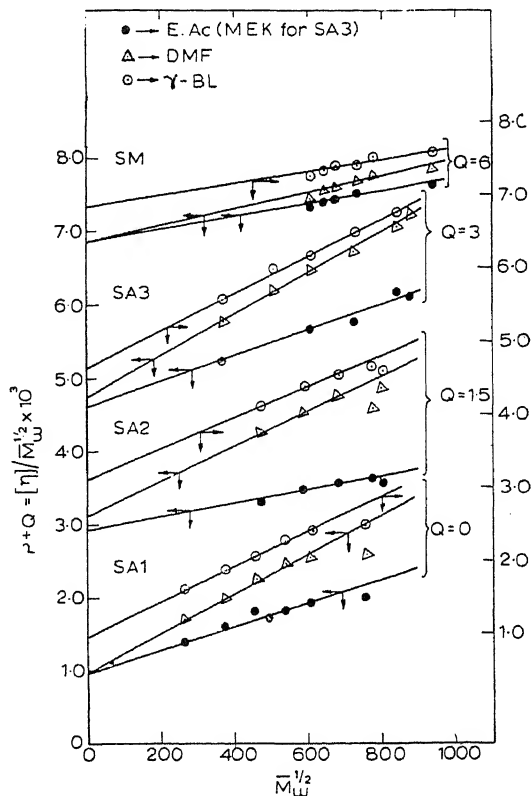


Fig. 1. S-F METHOD.

was evaluated and then  $\chi_1$  was calculated. Table I gives the data.

**Results and Discussions.**—Figure 2 shows the plots between  $[\delta_1^2/RT - \chi_1/V_1]$  and  $\delta_1$  for all the three copolymers in the solvents studied. The values of  $\delta_2$  obtained are  $10.4 \text{ (cal/cc)}^{1/2}$  for SA<sub>1</sub>,  $10.6 \text{ (cal/cc)}^{1/2}$  for SA<sub>2</sub> and  $10.9 \text{ (cal/cc)}^{1/2}$  for SA<sub>3</sub>. The increase in the value of  $\delta_2$  with an increase in the AN content is quite small.

It is interesting to note that for butadiene acrylonitrile (i.e., BUNA N rubber) systems the value of  $\delta_2$  increases<sup>7</sup> from  $8.7$  to  $10.3 \text{ (cal/cc)}^{1/2}$  as the AN content increases from 15% to 39%. The change of  $\delta_2$  in this case is appreciable with the

TABLE I  
Evaluation of  $\delta_2$  (solubility parameters of copolymers)

Solvent	$\delta_1$ (cal/cc) <sup>1/2</sup>	$x_1$	$V_1$ cc/mole	$x_1/V_1$	$\delta_1^2/RT$	$[(\delta_1^2/RT) - (x_1/V_1)]$
Polymer SA <sub>1</sub>						
EAc ..	9.1	0.456	98.0	0.00470	0.1374	0.1324
DMF ..	12.1	0.444	76.8	0.00579	0.2430	0.2372
$\gamma$ -BL ..	12.6	0.450	76.2	0.00590	0.2590	0.2580
Polymer SA <sub>2</sub>						
EAc ..	9.1	0.476	98.0	0.00503	0.1374	0.1324
DMF ..	12.1	0.449	76.8	0.00585	0.2430	0.2372
$\gamma$ -BL ..	12.6	0.455	76.2	0.00597	0.2590	0.2580
Polymer SA <sub>3</sub>						
MEK ..	9.3	0.4605	89.56	0.00514	0.1480	0.1379
DMF ..	12.1	0.4390	76.8	0.00510	0.2480	0.2372
$\gamma$ -BL ..	12.6	0.4460	76.2	0.005852	0.2590	0.2580
Polymer					$\delta_2$ (cal/cc) <sup>1/2</sup>	
SA <sub>1</sub>					Slope	
SA <sub>2</sub>					0.03465	10.4
SA <sub>3</sub>					0.03526	10.6
SA <sub>3</sub>					0.03612	10.9

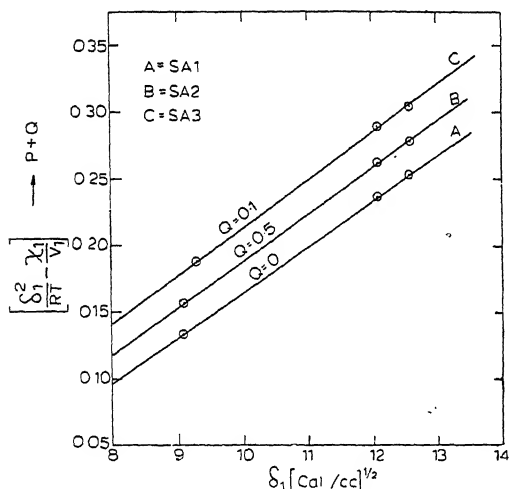


FIG. 2 EVALUATION OF  $\delta_2$

change in AN content. However, the change in the value of  $\delta_2$  for SA<sub>1</sub>, SA<sub>2</sub> and SA<sub>3</sub> for which the change in AN content is from 27.4 to 47.5 mole %, is not much. Also the value of  $\delta_2$  for the copolymer of lowest AN content in these series is high in comparison with the  $\delta_2$  value of polystyrene, i.e.,  $\delta_2 = 9.1$  (cal/cc)<sup>1/2</sup>. The estimated values of  $\delta_2$  of the copolymers help in the suitable choice of the solvents although the calculation of  $\delta_2$  by an absolute method is desirable.

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#### REACTIONS OF SOME UNSATURATED COMPOUNDS WITH 1, 3-DIAZA-2-PHOSPHOLIDINES AND -BOROLIDINES

DURING the course of our study on the insertion reactions of unsaturated compounds of the type  $A=B$  into the E-N bonds ( $E=Si, P, B$ ), we have previously reported the reactions of few unsaturated substrates like carbon disulphide, isocyanates and chloral on 1,3-diaza-2-silacyclopentanes<sup>1</sup>, 1,3-diaza-2-phospholidines<sup>2</sup> and 1,3-diaza-2-borolidines<sup>2,3</sup>, which produced either seven or nine-membered cyclic compounds (Fig. 1). Such

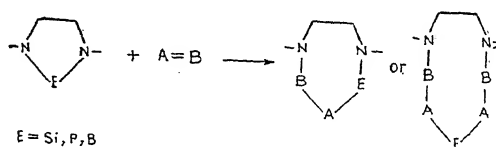


FIG. 1

insertion reactions are significant in providing easy syntheses for a wide variety of organometallic compounds, especially those having functional groups, and those which are not accessible or difficultly accessible by alternative routes<sup>4</sup>. The unsaturated compounds of the type  $A=B$  have some charge separation depending on the differences in the electronegativities of A and B; so also is with the E-N bonds. Such insertion reactions were postulated to proceed *via* a four-centre transition state<sup>1</sup> (Fig. 2) in steps of one molecule of  $A=B$  at a

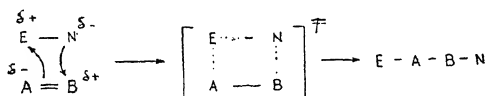


FIG. 2

time. Sometimes, the insertion of the first molecule probably deactivates the product towards further addition. As an extension of our previous work, the reactions of unsaturated compounds like isothiocyanates, carbon dioxide and sulphur dioxide with diazaphospholidines and diazaborolidines have been investigated and the results are reported here.

The reaction of phenyl isothiocyanate,  $C_6H_5NCS$ , with 1,3-dimethyl-2-phenyl-1,3-diaza-2-phospholidine in benzene in the mole ratio 2 : 1 produced a blood-red coloured liquid over 24 hr at room temperature. The reaction was slightly exothermic, and the same product could be obtained on refluxing the reactants in benzene for 3 hr. On removal of the solvent under vacuum, a deep-red liquid residue remained which corresponded to neither 1 : 1 nor 2 : 1 addition product. Similarly coloured products were also obtained when the isothiocyanate and the diazaphospholidine were allowed to react in the mole ratios 1 : 1 and 0.5 : 1; infrared spectra of all the products thus obtained showed a strong absorption band at  $2120\text{ cm}^{-1}$ , characteristic of CN stretching; distillation of the product under reduced pressure ( $130^\circ/1\text{ mm}$ ) yielded a distillate having an infrared absorption band at the same frequency as above. However, under similar conditions phenyl isothiocyanate yielded a 1 : 1 reaction product with 1,3-dimethyl-2-phenyl-1,3-diaza-2-borolidine producing a seven-membered ring compound<sup>2</sup>.

Reactions of  $CO_2$  with 1,3-dimethyl-2-phenyl-1,3-diaza-2-phospholidine and -borolidine were carried out by bubbling  $CO_2$  gas through benzene solutions of the heterocyclic compounds, and light yellow viscous products were obtained after the removal of the solvent under reduced pressure, which showed infrared bands at  $1710$  and  $1690\text{ cm}^{-1}$  respectively due to ketonic CO groups. Similarly,

on passing  $SO_2$  through a benzene solution of the above diazaphospholidine and on removal of the solvent under reduced pressure, deep-brown liquid product was obtained, which showed a very sharp band at  $1280\text{ cm}^{-1}$  due to probably thionyl type SO group. Although the analytical data of the product did not correspond to compounds of mole ratios of 1 : 1 or 1 : 2, reactions had certainly taken place as indicated by the nature of the products and by the presence of new  $\nu(CO)$  and  $\nu(SO)$  bands different from those of molecular  $CO_2$  and  $SO_2$ . As the reaction products of  $CS_2$  with diazaphospholidine<sup>2</sup> and *bis* (dimethylamino) phenylphosphine<sup>5</sup> were red coloured substances and as the insertion of  $C=S$  moiety in P-N bond had taken place<sup>2</sup> to produce new P-S linkages in those compounds, the red colouration might be attributed to the formation P-S linkages. Similarly, based on the observation that the reaction of phenyl isothiocyanate with the diazaphospholidine produced a blood-red coloured product which showed  $2120\text{ cm}^{-1}$  infrared band due to  $\nu(NC)$  irrespective of the ratio in which they were allowed to react, it may be suggested that the insertion of  $C=S$  bond rather than  $N=C$  in the P-N bond had taken place.

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#### OXIDIMETRIC ANALYSIS OF SOME THIOCYANATO COMPLEXES OF 3d-TRANSITION METALS, USING IODINE MONOCHLORIDE

THE polyhalide ion,  $ICl_2^-$  has been shown to be a convenient oxidant for the determination of many sulphur compounds, by excess-back titration methods<sup>1-4</sup>. It is now observed that this oxidant may be conveniently employed for the determination of thiocyanato complexes. In this communication we wish to report on the oxidation of six typical thiocyanato complexes.



**Complexes.**—The pyridine-thiocyanato complexes of Mn, Ni, Cu and Zn were prepared by standard methods involving the addition of pyridine to a mixture of aqueous solutions of thiocyanate and the respective metal sulphates<sup>5</sup>. The tetrathiocyanato mercurates of Co and Zn were prepared by the methods reported in the literature<sup>5</sup>. The precipitates were dried as recommended and their purities were checked by elemental analysis for metal, N and S.

Stock decimolar solutions of ICl in 5 N HCl were prepared and standardized as described earlier<sup>1,3</sup>. To measured volumes (20 ml) of the ICl solutions weighed samples of the complexes were added and the mixtures were thoroughly shaken from time to time. After allowing to stand for various time intervals (see Table I), the unreacted oxidant was determined by adding 10% aqueous KI (20 ml) and titrating the liberated iodine with standard thiosulphate.

**Results and Discussion.**—Typical results are presented in Table I. It may be seen that 6 equivalents of the oxidant are consumed per thiocyanate ligand, in agreement with the following equation :

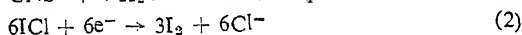
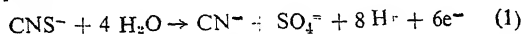


TABLE I

*Oxidation of thiocyanato complexes with Iodine monochloride*

No.	Complex	Amount of complex taken (mmol)	Time in hours	Amount of complex found (mmol)	% error
1	Mn(py) <sub>3</sub> (SCN) <sub>2</sub>	0.03831	1	0.03841	+0.26
2	"	0.04529	2	0.04539	+0.22
3	Ni(py) <sub>4</sub> (SCN) <sub>2</sub>	0.03811	1	Incomplete oxidation	
4	"	0.04056	2	0.04049	-0.17
5	Cu(py) <sub>2</sub> (SCN) <sub>2</sub>	0.06578	1	Incomplete oxidation	
6	"	0.05631	2	0.05601	-0.53
7	Zn(py) <sub>2</sub> (SCN) <sub>2</sub>	0.05509	1	Incomplete oxidation	
8	"	0.06866	2	0.06889	+0.34
9	Co[Hg(SCN) <sub>4</sub> ]	0.02380	$\frac{1}{2}$	0.02381	+0.04
10	"	0.05024	1	0.05003	-0.42
11	Zn[Hg(SCN) <sub>4</sub> ]	0.02098	$\frac{1}{2}$	0.02093	-0.24
12	"	0.02370	1	0.02363	-0.30

It may be seen from Table I that the thiocyanato mercurates are completely oxidized in about 15 minutes' time, whereas the pyridine-thiocyanate complexes require about 2 hours for quantitative oxidation. Longer reaction durations do not lead to increasing consumption of the oxidant. The apparent discrepancy in the case of Cu complex

(where only 5.5 moles of ICl appear to be consumed instead of the expected<sup>6</sup>) is easily explained because Cu, which is in the +2 oxidation state in the complex, gets reduced to the +1 oxidation state (as CuI) after the iodometric estimation.

Finally we wish to report that detailed studies of the oxidation of these complexes with 3 other oxidants, *i.e.*, Chloramine-T, Dichloramine-T and Dibromamine-T, were also carried out. The results with Dichloramine-T and Dibromamine-T were not analytically useful. Chloramine-T does oxidise all these complexes quantitatively where 8 equivalents of the oxidant are consumed per mole of thiocyanate ligand. However, oxidation of the complexes with Chloramine-T was found to be much slower than with ICl; in the case of the Zn complex, for example, the reaction mixture had to be left overnight (12 hours) for quantitative oxidation.

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## NOTES ON ABNORMAL SHRIMPS AND PRAWNS

MORPHOLOGICALLY abnormal specimens encountered in otherwise normal natural stocks of animals have always attracted the attention of biologists. In most cases, the origin of such abnormalities can be traced to injury or to some disturbance during early development, rather than to genetic causes.

During the course of our investigations on the shrimps and prawns of the estuarine system of river Krishna, we have come across a series of morphologically bizarre specimens in different species; there is no evidence that the abnormalities which they show have been recorded earlier.

*Macrobrachium equidens* (Dana): berried female, CL 16 mm. August 28, 1973; Nizampatnam. This specimen is totally devoid of the free rostrum (Fig. 1a); instead, there is a very short pointed extension of the anterior margin of the carapace, mid-dorsally. For some distance on either side of this, the edge of the carapace is fringed with a row of setae. In normal specimens, behind the base of the free rostrum, there are three teeth on the dorsal side of the carapace. In this specimen,

however, the anteriormost of the three teeth is represented by a patch of very short setae. The absence of the rostrum might be due to a mutation.

*Macrobrachium rude* (Heller): male, TL 69 mm (CL 15.5 mm), November 12, 1973, Guntur fish market. The fingers of second pereopod of right side are subequal (Fig. 1 b). However, both the second pereopods are of equal length: 42 mm.

*Macrobrachium malcolmsonii* (H. M. Edwards): female, TL 52 mm (CL 11 mm), November 15, 1973, Guntur fish market. The telson bears only one spine (on the right side) in the place of the usual two pairs of dorsal spines (Fig. 1 c).

*Macrobrachium rosenbergii* (De Man): male, TL 92 mm (CL 20.5 mm), November 12, 1973, Guntur fish market. The part depicted (Fig. 1 d) is the outer margin of the right uropodal exopod. Typically, this outer margin ends in a marginal tooth at the base of which is a single moveable spine, but it is not uncommon to find two spines, as depicted in the figure, in this species as well as in *M. malcolmsonii*.

*Leptocarpus potamiscus* (Kemp): two males, TL 36 mm (CL 6.5 mm) and TL 34 mm (CL 6 mm), July 29, 1973, near Nizampatnam. In the former specimen the telson is forked (Fig. 1 e); the left half is thin and appears to represent an extraneous growth.

In the latter specimen the forked telson is more complex (Fig. 1 f): the right half is thin and transparent while the left half is stout and tumorous. It would appear that following forking, one half was subjected to uncontrolled growth.

*Palaemon (Nematopalaemon) tenuipes* (Henderson): female, TL 62.5 mm (CL 11.5 mm), March 16, 1974, near Nizampatnam. This also appears to be a case of telson forking in which the right half of the fork has broken near its base (Fig. 1 g). The left half, with its two pairs of minute dorsal spines, has the appearance of a normal telson.

*Parapenaeopsis stylifera* (H. M. Edwards): female, TL 125 mm (CL 30 mm), December 24, 1973, near Nizampatnam. This is again a case of telson forking. The left fork has two pairs of fixed spines while the right fork has only one pair (Fig. 1 h). Typically, the telson bears two pairs of fixed sub-terminal spines, but it is not uncommon to come across adults with the proximal pair reduced and moveable, or even absent.

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## HETEROGONADISM IN THE PIGEON (*COLUMBA LIVIA* GMELIN)

HETEROGONADISM has not been reported to occur in nature-dwelling birds. A type of hereditary hermaphroditism, however, has been described in a special genetic race of pigeons where more than 80% of the adult males showed hermaphroditic features<sup>1,2</sup>. In extreme instances of such male pigeons the left gonad was an ovotestis and contained small and medium sized ovocytes; a complete left oviduct was also present. In males where both the testes were present the left testis was smaller than the right indicating an obvious persistence of the left cortex for a considerable length of time in this particular race of pigeons. Experimentally, various degrees of feminization of the male gonad have been induced by injections of oestrogen to allantois of 5-day chick embryos. In this case also, an ovotestis was obtained where the testicular medulla had partially regressed and the cortex showed various degrees of development<sup>3</sup>.

While studying the annual reproductive cycle of some avian species inhabiting the semi-arid and arid tracts of Rajasthan one specimen of the blue rock pigeon was encountered which exhibited heterogonadism. Out of about 5,000 pigeons examined

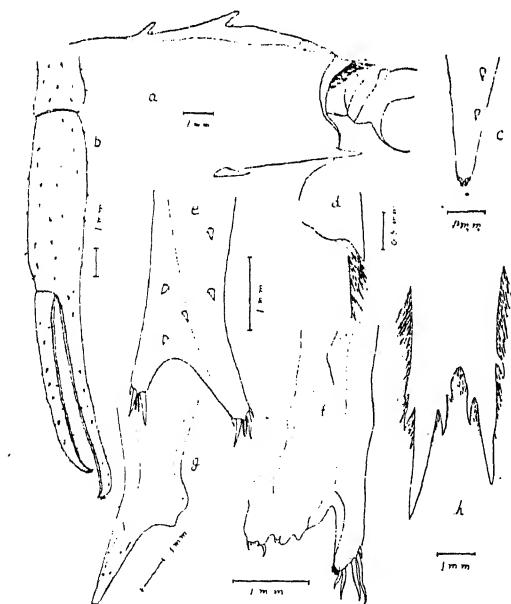
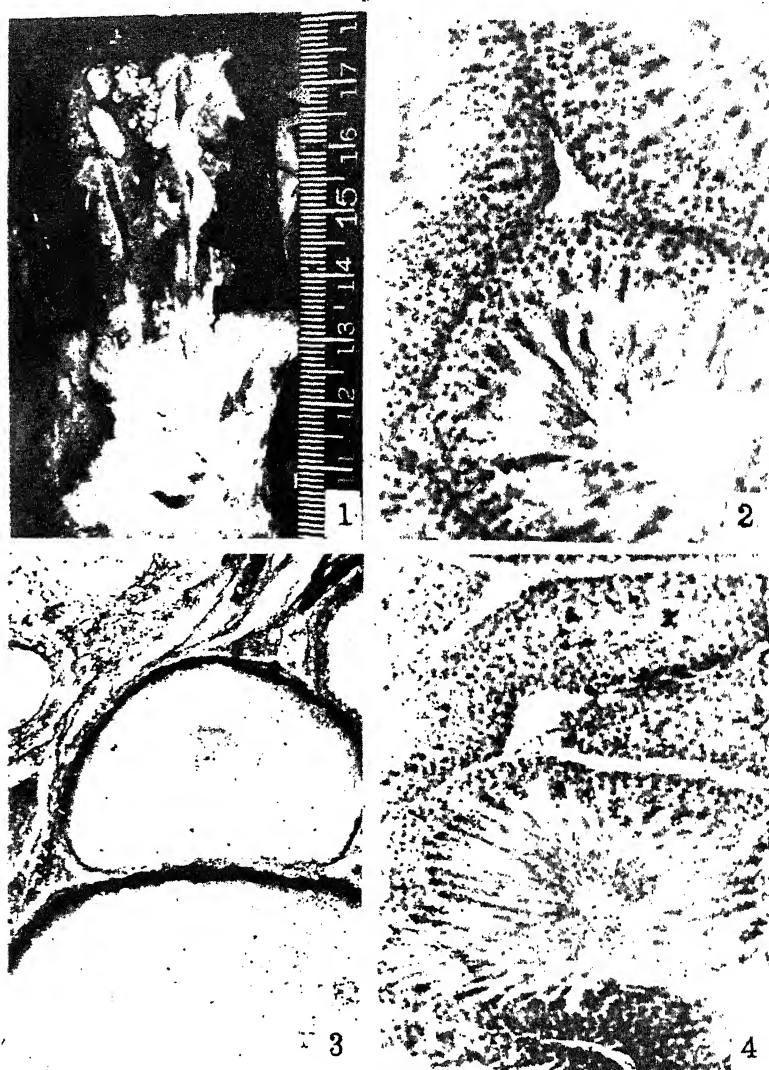


FIG. 1. a. *Macrobrachium equidens*; b. *M. rude*; c. *M. malcolmsonii*; d. *M. rosenbergii*; e. and f. *Leptocarpus potamiscus*; g. *Palaemon (Nematopalaemon) tenuipes*; h. *Parapenaeopsis stylifera*.

from the wild only one specimen exhibited this type of gonadal abnormality.

sperms (Figs. 2, 4) and resembled in every respect the testes of normal active males dissected on the



FIGS. 1-4. Fig. 1. Reproductive tract of the heterogonadic pigeon showing both the testes and ducts, left ovary and oviduct (scale in mm). Fig. 2. Cross section (c.s.) of the left testis of the abnormal pigeon showing sperms and other normal developmental stages. Haematoxylin-eosin (HE),  $\times 150$ . Fig. 3. C.S. of the left ovary of the abnormal pigeon showing well-developed ovarian follicles, HE,  $\times 150$ . Fig. 4. C.S. of the right testis of the abnormal pigeon showing sperms and other normal developmental stages, HE,  $\times 150$ .

The breeding season of the pigeon is from September to May, and in the remaining months the birds remain sexually inactive. The abnormal specimen was obtained from the wild in March. Autopsy of this specimen revealed, besides the two well-developed testes with their normal ducts, the presence of an apparently normal left ovary and a left oviduct. Both the testes showed motile

sperms (Table 1). As in the normal males, the left testis was larger than the right in this specimen also.

The left ovary of the abnormal specimen showed a large number of follicles (Fig. 3) which resembled in gross morphology and weight (Table 1); the ovarian follicles of the normal active females dissected on the same day. The size and the

TABLE I  
Size and weight of testes, ovary and oviduct in normal and heterogonadic pigeon

Specimen	Weight of the bird in gm	Testis				Ovary		Oviduct	
		Size in mm		Weight in gm		Mean diameter of largest follicle in mm	Mean ovarian weight in gm	Mean length in cm	Mean weight in gm
		Left	Right	Left	Right				
Normal male (35)*	309.5	21.0 × 8.0	17.0 × 9.0	0.618	0.590	..	..	..	..
Normal female (39)*	260.0	..	..	..	..	4.0	0.486	18.0	1.263
Heterogonadic pigeon (1)	248.5	18.0 × 9.0	15.5 × 7.0	0.733	0.726	4.5	0.486	13.0	0.467

\* Number of birds autopsied with the abnormal specimen.

weight of the oviduct of this abnormal specimen were much less than those of the normal active oviduct of the pigeons at this phase of reproductive activity. To my knowledge, such a type of heterogonadism has not been reported in pure breed of birds. It is not clear why this type of gonadal abnormality occurs in nature. It may be due to hormonal imbalance during the developmental stages of testes and ovary.

I am grateful to Prof. L. S. Ramaswami for guidance and encouragement during the course of this work.

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#### ROLE OF *HOPOLOAIMUS INDICUS* ON THE SEVERITY OF SEEDLING BLIGHT OF RICE

THE lance nematode, *Hoplolaimus indicus* Sher, 1963 has been reported as parasitic on roots of rice in uplands and well-drained soils<sup>1,2</sup>. At points of entry of the nematodes into roots, the seedling blight pathogen *Sclerotium rolfsii* Sacc. was frequently observed<sup>3-6</sup>. A study was undertaken to assess the role of the lance nematode in the spread of the seedling blight disease under inoculated conditions.\*

Water suspension of the nematodes was treated with 0.03% aureofungin solution. Mycelial suspension of the fungus was prepared from 4-day-old growth of *S. rolfsii* on rice grain medium in 250 ml conical flasks. Hulled seeds of the rice variety *Pankaj* were germinated under aseptic conditions.

Sterilised soil (50 g) was filled in each of 25 Petri plates (10 cm diam) and 8 ml distilled water was added to puddle the soil. The following treatments were given at the time of puddling after which 20 germinated seeds of *Pankaj* were sown per dish with five replications.

- (i) Control—no treatment.
- (ii) Sowing done after mixing 1,000 nematodes in the soil.
- (iii) Sowing done after mixing 10 ml mycelial suspension in the soil.
- (iv) Sowing done after mixing 1,000 nematodes + 10 ml mycelial suspension in the soil.
- (v) Same as (iv) but mixture of mycelial suspension + nematodes incubated for 16 hr at 28–30° C before incorporating in soil.

The germinated seeds were grown at  $25 \pm 1^\circ \text{C}$  in a growth chamber for 10 days when the mortality and disease symptoms in the seedlings, viz., yellowing or white striping or total whitening of the leaves and finally wilting or white radiating fungal growth and formation of sclerotial bodies at the base of the seedlings, were recorded.

The disease symptoms appeared in the seedlings in the treatment, (iii), (iv) and (v) and the mortality of seedlings ranged from 1 to 80% in the five treatments (Table I). Analysis of the angular values of the percentage survival of seedlings showed that nematodes alone did not cause mortality of seedlings whereas the fungus alone did. When nematodes were allowed to mix with the fungus for 16 hours prior to inoculation, there was higher mortality of seedlings as compared to the freshly combined inoculum of the nematode and the fungus or the fungus alone. The higher mortality of the seedlings in the treatment (v) might be due to the fact that the longer incubation might have enabled the fungus to adhere to the surface of the nematode better than in the case of the freshly

TABLE I

Survival of seedlings of rice variety Pankaj inoculated with the lance nematode (*Hoplolaimus indicus*) alone and in combination with the seedling blight pathogen (*Sclerotium rolfsii*) (Means of five replicates)

Treatment to soil prior to sowing of sprouts	Survival of seedlings		Mortality of seedlings (percentage)
	Percent-age	Angular values	
(i) Control	99	87.4	1.0
(ii) Nematode alone	99	87.4	1.0
(iii) Fungus alone	42	40.4	58.0
(iv) Nematode + Fungus mixture	36	36.8	64.0
(v) Nematode + Fungus mixture incubated for 16 hr prior to inoculation	20	25.6	80.0
C.D. 0.05	..	8.29	..
0.01	..	11.29	..

combined inoculum of the nematode and the fungus. It would appear that the nematodes not only have provided points of entry for the fungus but also have passively carried the pathogen on their body surface into the root tissues, thus causing enhancement of the mortality of the seedlings. Further studies to understand this association are in progress.

Association of the root lesion nematode (*Pratylenchus brachyurus*) with *Sclerotium rolfsii* causing Southern Blight of peanuts<sup>7</sup>, of *Trichodorus* sp., *Pratylenchus* sp., *Helicotylenchus* sp. and *Aphelenchoides* sp., with *Pellicularia rolfsii* causing wilt in ragi (*Eleusine coracana*)<sup>8</sup> has been established. This is the first report on the role of parasitic nematode *Hoplolaimus indicus* in the enhancement of the seedling blight disease of rice incited by *S. rolfsii*.

The senior author is grateful to the Indian Council of Agricultural Research for the award of a Senior Research Fellowship for conducting research on the lance nematode. The authors are grateful to Dr. S. Y. Padmanabhan, Director, Central Rice Research Institute, Cuttack, for encouragement and for providing research facilities. Division of Entomology and K. V. RAMANA. Division of Plant Pathology, S. C. MATHUR. Central Rice Res. Institute, Y. S. RAO. Cuttack 753006, India, March 16, 1974.

\* Part of Ph.D. thesis submitted by the senior author to the Orissa University of Agriculture and Technology, Bhubaneswar.

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# FIRST RECORD OF THE GREGARINE *FARINOCYSTIS TRIBOLII* WEISER ON *TRIBOLIUM CASTANEUM* HERBST FROM INDIA

WEISER (1953) was the first to record the schizogregarine *Farinocystis tribolii* on *Tribolium castaneum*. Dissanaik (1955) described the same gregarine as *Tribollocystis garnhami*. Ashford (1968) confirmed the synonymy of *T. garnhami* Dissanaik with *F. tribolii* Weiser.

In a laboratory culture of *T. castaneum* in the Agricultural College and Research Institute, Coimbatore, a few dead grubs were observed. Tissue smears in saline, on examination by phase contrast, revealed the spores of *F. tribolii* (Fig. 1). This appears to be the first record of the pathogen on *T. castaneum* in India.

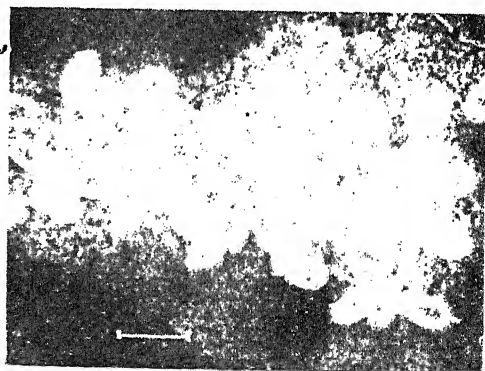


FIG. 1. Spores of schizogregarine, phase contrast, *Farinocystis tribolii* in *Tribolium castaneum*. Line, = 16  $\mu$ .

The spore suspension in distilled water was found to be infective on inoculation to disease-free grubs of *T. castaneum*. Infected grubs somewhat whitish in colour could be observed on the surface of the diet. On piercing the infected grub with a sharp needle, whitish fluid exuded which was found to be full of spores. Fifty spores measured from 12.00  $\mu$  to 14.40  $\mu$  by from 6.40  $\mu$  to 8.00  $\mu$ . Marshall Laird (1959) reported the measurements of 50 spores of *T. garnhami* to be from 13.3  $\mu$  to 14.3  $\mu$  by from 6.7  $\mu$  to 7.8  $\mu$ .

Histopathological studies revealed the spores and other stages of the parasite in the fat body of the host insect.

The authors wish to express their gratitude to Dr. Jean R. Adams and Dr. Goodwin of Insect Pathology Laboratory, Beltsville, Maryland, for their help in the identification of the pathogen.

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#### OCCURRENCE OF SOYBEAN YELLOW MOSAIC IN UTTAR PRADESH

SOYBEAN has been reported to be susceptible to a number of virus diseases, most common among those are soybean mosaic, yellow mosaic and bud blight. A survey of virus diseases of soybean was made in 1971 and 1972 through the important soybean growing areas of Uttar Pradesh. The areas included hill regions of Almora, Pithoragarh and hills and tarai regions of Nainital District. In other parts of Uttar Pradesh, soybean is not yet a favourite crop but it is grown in private allotments in and around Gorakhpur and these were also surveyed. Soybean was seen infected with yellow mosaic in every area surveyed, except in the hills. Its incidence was 30-40% in tarai regions and 20-30% in Districts of Eastern Uttar Pradesh.

The earlier reports (Conover<sup>3</sup>, Castellani<sup>4</sup>, Quantz<sup>5,6</sup>) on yellow mosaic of soybean show that this disease causes considerable loss to the yield and quality of grains. This disease may reduce the yield from 5-70% depending upon the varieties. Considering the economic importance of this disease the present investigation was undertaken leading the growers towards its control.

In the fields soybean plants infected with yellow mosaic disease were slightly stunted and produced only a few branches on them. The leaflets of these plants were slightly reduced in size and became narrower as compared to those of healthy plants. The infected leaflets showed bright, yellowish patches scattered throughout the lamina, without curling or distortion (Fig. 1). Infected plants possessed lesser pods on them which were very much reduced in size. The pods from such plants bore only one or two seeds instead of three seeds in normal.

The virus could be transmitted only to the plants of family Leguminosae, viz., *Glycine max* (L.) Merr., *Phaseolus mungo* L., *P. aureus* Roxb., *P. vulgaris* L. and *Vigna sinensis* L.



FIG. 1. Yellow mosaic disease in soybean (local variety). Healthy leaf on left.

The virus was not transmissible through sap inoculation. The aphids, *Myzus persicae* Sulz., *Aphis gossypii* Glover, and *A. craccivora* Koch could not transmit the disease. Seed transmission also was not achieved. Transmission was achieved only with white fly (*Bemisia tabaci* Gennadius). White fly culture was maintained on *Phaseolus aureus* plants during October–November. The healthy potted seedlings of *P. aureus* with white flies were kept in a cage and were allowed to multiply there. Young flies were taken for transmission experiments.

Transmission experiments were performed in glass transmission tubes (10 × 2.5 cm) open on both the ends. One end of the tube was tied with a piece of nylon cloth and the other end was fitted with a splitted cork having a circular hole in the centre, through which white flies were released in the tube and tip of the healthy seedling was inserted. A plastic aspirator<sup>1</sup> was used for handling and transferring white flies during transmission experiments.

The flies were given a pre-acquisition fasting of 6 hr and acquisition feeding of 2 hr followed by an infection feeding of 24 hr. Ten flies were released in each transmission tube. After infection feeding the flies were removed and the plants were sprayed and kept inside insect proof glass house.

Twenty healthy seedlings of soybean were used in the transmission experiment. The seedlings showed typical yellow mosaic symptoms after about 20 days of treatment. The transmission was only 60%. The same treatments were given to 20 seedlings of each of the following plants: *Phaseolus*

mungo, *P. aureus*, *P. vulgaris*, and *Vigna sinensis* Savi. The percentage transmission in these species was lesser (30–40%) than in soybean.

Pierce<sup>2</sup> was the first to report yellow mosaic disease of soybean caused by Bean virus 2 (yellow bean mosaic virus). Conover<sup>3</sup> gave a full account of two viruses causing mosaic disease on soybean, namely, Soja virus 1 (soybean mosaic virus) and *Phaseolus* Virus 2 (bean yellow mosaic virus). Castellani<sup>4</sup> recorded the symptoms, manner of spread and control of soybean mosaic virus, yellow mosaic of soybean (bean yellow mosaic virus) and bud blight (tobacco ring spot virus). Quantz<sup>5,6</sup> recorded soybean among the natural hosts of bean yellow mosaic virus and presented an information on symptomatology, host range, transmissibility and control of this virus. Soybean crop was found to be affected by Mung yellow mosaic virus in the farms of G. B. Pant University<sup>7</sup>. The virus could not be transmitted mechanically. Similarly, no seed transmission was obtained. The virus could be transmitted only by white fly (*Bemisia tabaci* Gennadius). The virus affected a number of plants including members of the family Graminae and Compositae.

The present virus resembles with mung yellow mosaic virus in symptomatology, host range and transmissibility.

Leguminous weeds like species of *Trigonella* growing in and around the fields of soybean crop were suspected to act as the source of yellow mosaic disease. These plants, on the other hand, are the host for white fly during the crop season (August–November). It is, therefore, concluded that white fly is an efficient vector spreading this disease from diseased to healthy plants in nature. Regular weeding out of the leguminous plants from soybean fields and surrounding plots may be taken as a step towards the control of this disease.

The author is deeply grateful to Dr. K. S. Bhargava for providing necessary facilities and to Dr. R. D. Joshi for his helpful suggestions.

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## HYBRIDIZATION IN MODERN ROSES

### III. Selfings in Control and Irradiated Plants

THE road to hybridize rose is paved with stumbling rocks still the number of rose cultivars is increasing day by day. American Rose Society alone has registered 1677 cultivars during the last decade (Allen, 1970). Bud sports have been an important source of evolution of a large number of roses (Hurst, 1941; Wylie, 1954, 1955 a, 1955 b; Pal, 1966). Many workers have been able to induce mutations in different rose cultivars with the help of neutrons, gamma-rays and x-rays (Lata, 1973). The effect of radiations on the breeding behaviour of roses was not studied by them. Present experiment was conducted to evaluate these effects, therefore, reciprocal pollinations were done among control and irradiated plants of seven rose cultivars.

### MATERIALS AND METHODS

Control and 3–5 Krads of gamma-ray treated plants of cultivars Caledonia, Oklahoma, Papa Meilland, Pink Parfait, Prelude, Quebec and Virgo were employed in the breeding experiments which were conducted during February–April, 1971. About fifty reciprocal pollinations were done between control and irradiated rose cultivars except where it did not seem to be effective and the fruits failed to develop. Data on selfings and intervarietal crosses in cultivars Pink Parfait, Quebec and Virgo was also incorporated to compare the breeding behaviour in these good seed setting garden roses. Results on percentage of fruit set, seed germination and average number of seeds per fruit on above-mentioned three cultivars have been presented in Table I. Rest of the cultivars were not included in the table since the data were scanty on account of poor fruit set. Ripened fruits were collected and stored at low temperature. Seeds were sown on November 21, 1971 following the usual technique (Lata, 1971).

Data on successful pollinations, number and percentage of fruit set and number of seeds per fruit were recorded. Observations were made on the number and percentage of seeds germinated. A record on growth habit and flowers produced from each seedling was also kept.

### OBSERVATIONS

No fruit set was observed in cultivars Caledonia and Papa Meilland. Cultivar Prelude also gave very poor fruit set, so much so that only one fruit developed in irradiated × non-irradiated pollination. This fruit consisted of 9 seeds which failed to germinate.

In Oklahoma no fruit set was observed when selfing was practised among non-irradiated plants.

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However, in pollinations using control  $\times$  irradiated combination, former being the female parent, the fruits failed to develop. In reciprocal pollinations 36% cases were successful. The number of seeds per fruit was low. Out of three seeds sown only two germinated which failed to survive after about two months.

Selfings of non-irradiated Pink Parfait resulted in 66.6% fruit set (Table I). A higher percentage of fruit set, (76%) was recorded when the emasculated control flowers were pollinated with the pollen from irradiated flowers. The average number of seeds per fruit was more in the latter case. In reciprocal pollinations among irradiated  $\times$  control flowers 72% fruit set was recorded. The average number of seeds per fruit, in three kinds of selfings, varied from 26.6% to 31.8%. Higher number of seeds per fruit was recorded in intervarietal crosses though the fruit set did not vary significantly. One hundred and five seeds from different selfings were sown which resulted in 11.4% germination. The germination was higher in the seeds resulted from intervarietal crosses. The flowers of different selfed seedlings were light to deep pink in colour.

vary significantly among the different selfings. The percentage of fruit set and the number of seeds per fruit was higher in intervarietal crosses. Only one seedling each from control  $\times$  irradiated and *vice versa* pollinations germinated which died before producing any flower. None of the seeds from non-irradiated selfings germinated.

Selfings in control plants of cultivars Caledonia, Oklahoma, Papa Meilland and Prelude failed to set fruits but better fruit set was observed in Oklahoma and Prelude when irradiated plants were used as female parent and the control as male. It seems that irradiation was probably helpful in restoring the female fertility in these cultivars. In general, better fruit set in reciprocal combinations between irradiated and control plants was recorded in cultivars Pink Parfait, Quebec and Virgo as compared to those where only non-irradiated plants were used. This again supports the assumption that irradiation has helped in restoring the fertility in garden roses probably by breaking the self-incompatibility barrier, to some extent. Lewis and Crowe (1954) have been able to induce self-fertility in some fruit trees by application of x-rays.

TABLE I  
*Selfing and crossing in certain good seed setting rose cultivars*

Sl. No.	Name of cultivars	Control $\times$ Control		Control $\times$ Irradiated		Irradiated $\times$ Control		Intervarietal crosses		% seed germination	
		Fruit set %	Av. No. seeds	Fruit set %	Av. No. seeds	Fruit set %	Av. No. seeds	Fruit set %	Av. No. seeds	Selfs	Crosses
	Pink Parfait	66.6	30.0	76.0	31.8	72.0	29.6	70.6	55.0	11.4	19.5
12	Quebec	20.0	2.5	28.0	3.4	16.0	3.0	80.0	43.0	0.0	10.0
3	Virgo	16.6	5.0	20.0	7.3	28.0	6.7	52.0	50.0	3.9	19.2

Selfing and crossing in certain good seed setting rose cultivars.

Selfing among non-irradiated Quebec flowers resulted in 20% fruit set (Table I). Better fruit set was observed in control  $\times$  irradiated pollinations than *vice versa*, i.e., 28% and 16% respectively. Average number of seeds per fruit was almost equal in the above-mentioned combinations. Percentage of fruit set and average number of seeds per fruit was enhanced in the intervarietal crosses. None of the seeds from different selfings germinated whereas 10% germination was recorded in the seeds obtained from crosses.

The emasculated control flowers of cultivar Virgo were pollinated with the pollen from control and irradiated plants which resulted in 16.6% and 20% fruit set respectively (Table I). About 28% fruit set was observed in irradiated  $\times$  non-irradiated combination. The number of seeds per fruit did not

Cultivars Pink Parfait, Quebec and Virgo gave a higher percentage of fruit set in intervarietal crosses as compared with the different selfings. Self-incompatibility resulted in poor fruit set in garden roses as compared with the crosses (Lata, 1971). According to Hurst (1969) complex hybridity accompanied with polyploidy was responsible for low fruit set in garden roses. Cultivar Oklahoma, Papa Meilland, Pink Parfait, Prelude and Virgo are polyploids and have arisen as hybrids of complex parents (McFarland, 1965). However the ancestry of Caledonia and Quebec is not known (McFarland, 1965) though they are allo-tetraploids (unpublished). It is interesting to note here that Chrysler Imperial  $\times$  Charles Mallerin were the parents of cultivars Oklahoma and Papa Meilland (McFarland, 1965). Wheatcroft (1967) reported



that the seedlings of the same cross may not be alike. Furthermore, on account of complex pedigree of garden roses new forms can be obtained even without resorting to artificial pollination (Pal, 1966).

In general the average number of seeds per fruit and seed germination in selfs was significantly lower than those procured from intervarietal crosses. The life span for seedlings obtained from selfed seeds was found to be relatively short as compared to the crossings. Selfed seedlings of Oklahoma and Virgo died even before producing a flower. A loss in vigour has been reported in various crop plants which was due to inbreeding depression. Wylie (1954), Eva (1968) and Lata (1971, 1972) have recorded this phenomenon in roses.

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National Botanic Gardens, PUSHPA LATA.  
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#### SYSTEMIC LESIONS OF SEED BORNE BRINJAL MOSAIC VIRUS IN SOME VARIETIES OF BRINJAL\*

BRINJAL mosaic virus (BMV) has been reported to occur on several varieties of brinjal from the Punjab<sup>5</sup>. In the identification studies it was found that this virus differed in physical properties, host reaction and transmission from the mosaic type of viruses reported on brinjal by several workers from India<sup>2-8-11</sup>. However, it possessed some similarities in its symptom expression, host range and seed transmission ability to the SS isolate of Sharma<sup>10</sup>. The most important feature of the

virus was its exclusive seed transmission and internal seed borne nature which was not known earlier. Infected seed constituted the major and only source of inoculum. The extent of seed transmission was upto 40% in some varieties. The ability to carry the virus in seeds and retention of active viral inoculum, varied greatly with the variety<sup>6</sup>.

During seed transmission studies of BMV in brinjal varieties, it was observed that plants raised from the infected seeds produced mosaic mottling symptoms characteristic of the disease (Fig. 1). However, a deviation from these usual symptoms was noticed on a few seedlings grown from stored infected seeds.



FIG. 1. Brinjal seedling showing typical systemic mosaic symptoms of BMV.

The seeds collected exclusively from infected, selfed plants of varieties Pusa Kranti, Pusa Purple Long, R-34 and S-5, stored in paper bags and kept at room temperature were periodically sown to observe the effect of storage on seed transmission. Though the virus activity was slowly decreased in storage (Table I), the mosaic mottling symptoms were the identifying feature of diseased seedlings upto 180 days of storage. However, instead of usual mosaic symptoms, systemic chlorotic lesions appeared on seedlings grown from infected seeds stored for 210 days (Fig. 2). The lesions were restricted to third and fourth leaf and did not appear on further leaves. They were oval to circular with a lighter centre in the beginning but

TABLE I  
Seed transmission of BMV after storage

Variety	Percentage of seed transmission after storage for (days)			
	30	120	180	210*
Pusa Kranti	41.30	22.64	7.63	1.68
R-34	33.13	26.15	13.67	2.57
S-5	19.00	14.36	9.21	2.00
Pusa Purple Long	35.32	21.15	5.51	2.08

\* Instead of usual mosaic symptoms, systemic chlorotic lesions appeared.

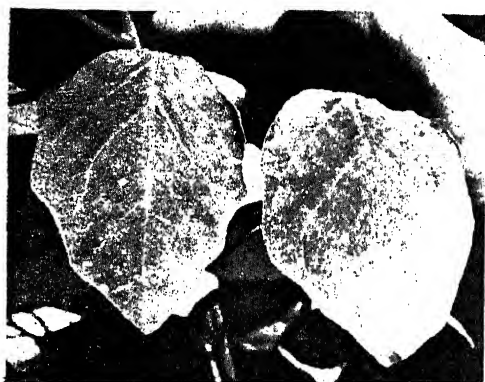


FIG. 2. Brinjal leaves showing systemic chlorotic lesions of BMV.

diffused after 4 to 5 days. The lesions were confirmed to be due to BMV by sap inoculating them on young seedlings of brinjal, using carborundum (600 mesh) as an abrasive, where they produced clear mosaic symptoms in 20 days. The absence of virus in the symptomless leaves was confirmed by similar test. Appearance of such lesions on plants raised from virus infected seeds has presently no analogy. Systemic necrotic lesions of tobacco mosaic virus (TMV) were observed on leaves of *Datura* stocks wedge-grafted with TMV-infected tomato scion<sup>7</sup>. Occasionally some strains of TMV also produced lesions systemically in few species of *Chenopodium*, where the virus was not seed-transmitted. It is also reported that the most common local lesion hosts like *Chenopodium amaranticolor* and *Gomphrena globosa* carried fan leaf and yellow mosaic viruses of grapes<sup>2</sup> and tomato ring spot virus<sup>4</sup> respectively, through their seeds. But in these cases the infected plants either were symptomless or exhibited mottling symptoms and never systemic lesions like those noticed in the present studies.

The effect of temperature was taken into consideration. It was observed that temperature influenced the incubation period of BMV but had no role

in the formation of systemic lesions. During lower temperatures of February–March (16.3° to 19.4° C) the symptoms appeared in 64–67 days while at higher temperatures of July–August (30.6 to 34.7° C), they appeared in 47–51 days in these varieties. The temperature (30.6 to 34.7° C) at which the systemic lesions appeared was also favourable for mosaic symptom expression and was evident from the fact that the chlorotic lesions after sap-inoculation produced clear mosaic symptoms on young brinjal seedlings during the same period.

According to Bos<sup>1</sup>, systemic lesions might be produced due to a low concentration of virus in the transported phloem contents giving rise to a localized reaction at those spots where infection units succeed in establishing new multiplication centres. The gradual inactivation of BMV in storage implies a low concentration of virus in the seeds which after transportation to the multiplication centres might have been insufficient to produce characteristic mosaic syndrome, thus resulting in localized lesions. The restriction of lesions to third and fourth leaf further strengthens the view that it was probably due to a low concentration of virus, systemic mosaic symptoms failed to occur on younger leaves of the plants.

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### HORMONAL REGULATION OF MANGO MALFORMATION

MANGO malformation (Floral) is widely distributed all over India and its incidence is more severe in north-west than in north-east or south. Recently this disease has also been reported from Pakistan<sup>1</sup>, Central America, Mexico and Israel<sup>2</sup>. The unique and visible symptom of this malady is the deformation of the panicle, which consists mainly in a shortening of the primary and secondary axes and thickened rachis giving the flower a characteristic clustered appearance (Fig. 1). Apical dominance is suppressed in malformed panicles which very seldom set fruits due to production of mostly male flowers. Deformed panicles ultimately dry up persisting on the tree for many months as black masses of dead tissues.



Fig. 1. Healthy (left) and malformed (right) panicles of mango.

Going through the two reviews on mango malformation it is clear that the biochemical knowledge of this malady has been rather poor<sup>3,4</sup>. Since auxins are involved in the phenomenon of apical dominance, an attempt has been made to find out the levels of auxin-like substances and inhibitors both in healthy and malformed buds of Dashehari mango, a popular cultivar of north India.

The experiment was conducted in the Experimental Orchard of the Indian Agricultural Research Institute, New Delhi, using 20-year old Dashehari tree in 1973. Samples with respect to healthy and malformed buds were collected at the bud burst stage (2 cm long). The extraction, purification of extracts for chromatography and bioassay were done as described by Kefford<sup>5</sup> with some modifications<sup>6</sup>. For bioassay of endogenous growth substances, the technique of Nitsch and Nitsch<sup>7</sup> was followed.

Growth promoting activities with respect to free neutral, free acidic, bound neutral and bound

acidic fractions of auxin have been presented in Fig. 2. With regard to free neutral fraction it was observed that in the chromatograms of healthy buds growth promoting regions were noted practically at all the Rf values except at Rf 0.7, where inhibition was observed. In the case of malformed buds, although two growth promoting regions one between Rf 0.1–0.4 and other between 0.8–1.0 were noted, their levels were lower than the healthy ones, specially at Rf 0.1, 0.3 and 0.4. Beside this, while a marked inhibitory zone was noted between Rf 0.5–0.7 in the case of malformed buds, the same was absent in the chromatograms of healthy buds excepting at Rf 0.7. This inhibitory zone contains physiological inhibitor and not just a toxic product is shown by the fact that first internode sections washed and transferred to distilled water after 24 hours incubation with inhibitor recovered to a certain extent.

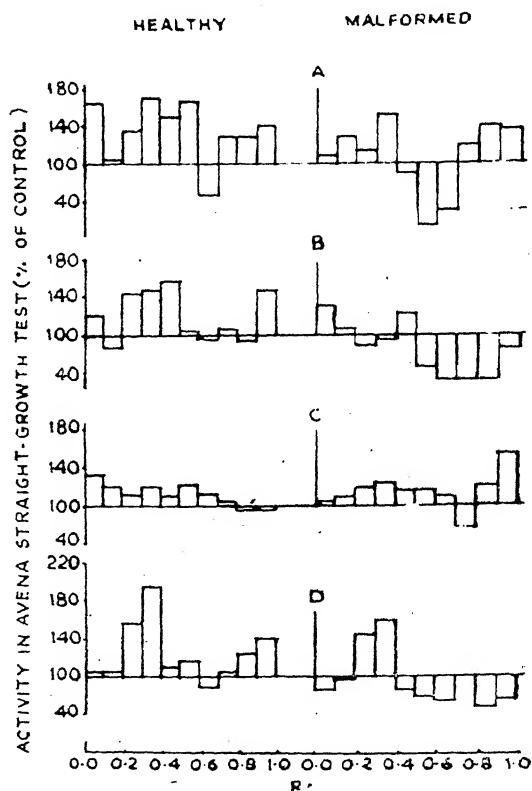


Fig. 2. Biological activity of free neutral (A), free acidic (B), bound neutral (C) and bound acidic (D) fractions separated, from healthy and malformed panicles of mango.

In the free acidic fraction of healthy buds the growth promoting activity was noted between Rf 0.3–0.4 which corresponds to the Rf of endo-

genous indoleacetic acid in isopropanol-ammonia-water (8:1:1 v/v). But this was completely lacking in malformed ones and instead of promotion, marked inhibition was noted in this region. Beside this, while a growth promoting region was noted at Rf 1.0 in healthy buds, in malformed buds a marked inhibitory zone was observed between Rf 0.5-1.0.

Bound neutral fractions did not show much changes in their biological activities as measured by *avena* straight growth test.

Chromatograms of bound acidic fractions extracted from healthy buds were characterized by extensive growth promoting regions in many cases, extending from the origin to the solvent front, excepting at Rf 0.7 where growth inhibiting activity was seen. In the case of malformed buds, two inhibitory regions one between Rf 0.1-0.2 and other between 0.5-1.0 were noted. Like healthy buds, the extracts of malformed buds also showed a bioactive compound between Rf 0.3-0.4 but its level was lower than the normal ones.

From the foregoing results it is clear that while the levels of all the four fractions of auxin are higher in healthy buds as compared to malformed ones, the levels of inhibitors showed a reverse picture in two types of buds. These results bring out the fact that mango malformation is controlled by the endogenous levels of growth regulating substances. Since there is imbalance between growth promoters and growth inhibitors, it resulted in the suppression of the apical dominance and made the developing panicles malformed. This hypothesis has, further, been confirmed by the fact that spraying of 200 ppm  $\alpha$ -naphthaleneacetic acid before fruit-bud differentiation reduces the incidence of malformation to the extent of 75%<sup>8</sup>.

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## DEVELOPMENT OF OVULE AND FEMALE GAMETOPHYTE IN *EOMECON CHIONANTHA* HANCE

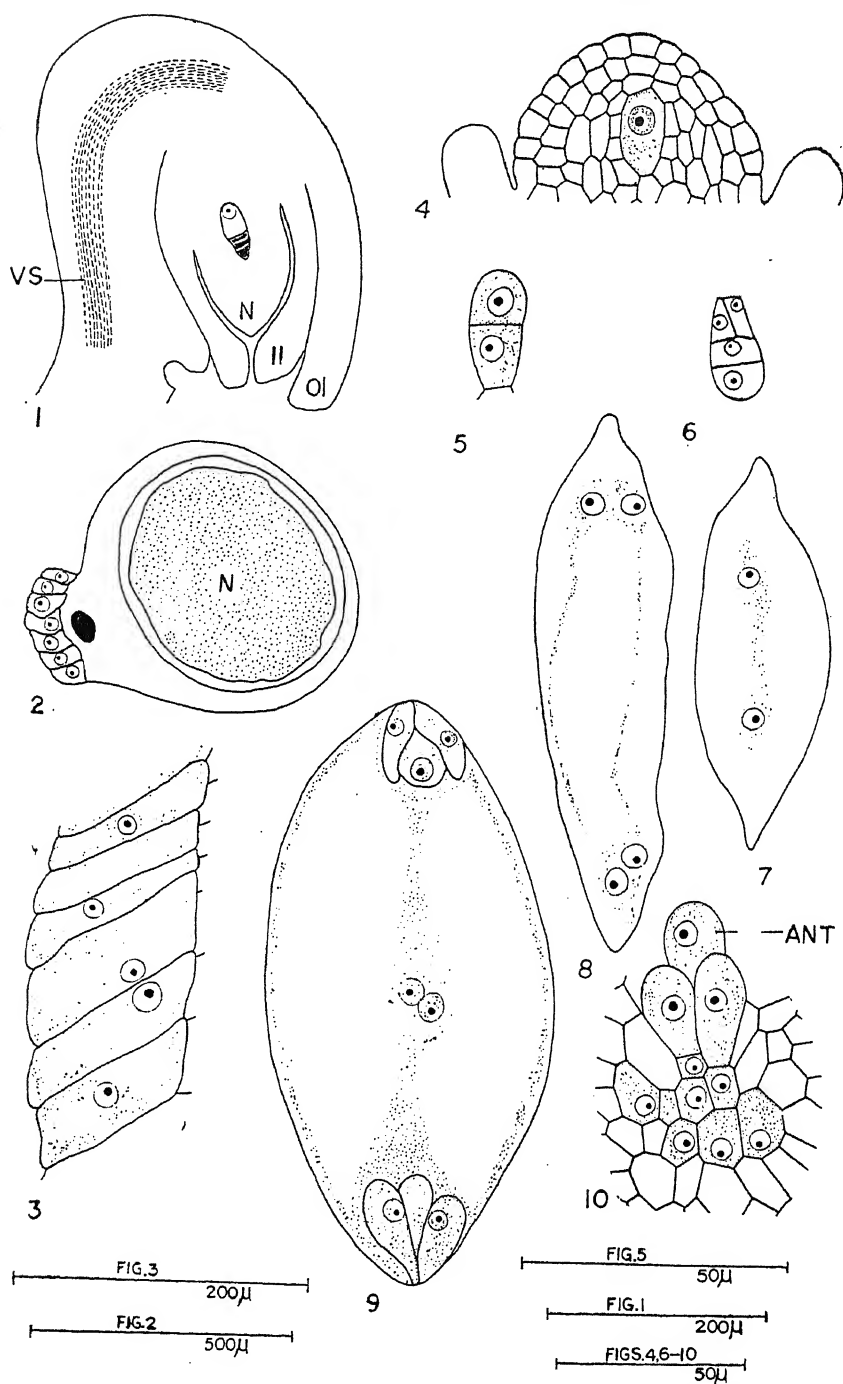
EARLIER embryological work on the family Papaveraceae to which *Eomecon chionantha* belongs has been reviewed by a number of workers<sup>1-6</sup>. The embryology of *Eomecon chionantha* has not been investigated so far and, therefore, an attempt has been made to study this species. The present account relates to the development of ovule and female gametophyte.

Flower buds and open flowers at different stages of development were collected from plants growing in the conservatory of the National Botanic Gardens, Lucknow. The material was fixed in FAA and preserved in 70% ethanol. Microtome sections were cut at 7-12  $\mu$  thickness and were stained in Safranin-fast green combination.

*Ovary and ovule*.—The ovary is superior, bicarpellary, syncarpous, unilocular with parietal placentation. Numerous ovular primordia develop as small lateral outgrowths on the placenta and soon they become anatropous (Fig. 1). The ovule is crassinucellar and bitegmal. The micropyle is formed by both the integuments which are initiated, more or less, simultaneously. The funicular vascular supply extends up to the chalaza (Fig. 1). A feature of interest is the elongation of the epidermal cells along the raphe in the form of a palisade layer (Figs. 2, 3).

*Megasporogenesis and megagametogenesis*.—The female archesporial cell differentiates in the hypodermal layer of the nucellus. It enlarges and divides periclinally to form a primary parietal cell and a megaspore mother cell. The former divides to push the megaspore cell deeper (Fig. 4) which soon enlarges and undergoes meiosis I forming a dyad (Fig. 5) and then a tetrad of megaspores, arranged either in a linear or T-shaped manner (Fig. 6). The chalazal megaspore functions while the other three degenerate. The nucleus of this megaspore undergoes three successive mitotic divisions forming the 2-nucleate, 4-nucleate and 8-nucleate embryo sacs (Figs. 7, 8, 9). The development of the female gametophyte thus follows the Polygonum type.

The egg apparatus consists of two synergids and the egg. The synergids and egg show the usual positions of nucleus and vacuole. The polar nuclei meet near the centre of the embryo sac. The antipodal cells enlarge considerably (Fig. 9) and the ovular tissue below it shows dense cytoplasmic cells (Fig. 10). Similar enlarged antipodals have been reported in other taxa of this family like *Argemone*<sup>2</sup>, *Fumaria*<sup>1-6</sup>, *Corydalis* and *Papaver*<sup>1</sup>.



Figs. 1-10. *Eomecon chionantha*. Fig. 1. L.s. ovule at the functional megaspore stage. Fig. 2. T.s. ovule showing palisade-like epidermal cells in the raphe region. Fig. 3. Palisade-like cells enlarged. Fig. 4. L.s. part of ovule showing embedded megaspore mother cell. Figs. 5-6. Dyad and tetrad of megaspore respectively. Figs. 7-8. 2- and 4-nucleate embryo sac respectively. Fig. 9. Organised female gametophyte. Fig. 10. L.s. part of ovule showing three antipodal cells and richly cytoplasmic cells below.  
(ANT, Antipodal cells; II, inner integument; N, nucellus; OI, outer integument, VS, vascular supply.)

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#### SUGAR AND NITROGEN CONTENT IN RICE AS INFLUENCED BY SHORT PHOTOPERIOD

RICE is principally a short-day plant. When exposed to short days it flowers considerably earlier than the control plants. That the induction of earlier

lings were exposed to photoinductive cycles each consisting of 8 hr photoperiod alternated with 16 hr dark period. The second set of plants were grown under natural photoperiodic cycle where the seedlings remained vegetative under the average day-length of 13-13 hr prevailing during the period of study, June-July, the normal rice growing season in India. The seedlings had a minimum of 4 active green leaves at the treatment initiation. Clean, weighed fresh leaf (4th leaf which was found active and healthy till the termination of the treatment) was collected from control and treated seedlings at the age of 29, 31, 33, 35, 37 and 39 days for estimation of sugars and nitrogen fractions following respectively Somogyi's method (1945) and Microjeldhal method as was used by Markham (1942). The control seedlings were purely vegetative during the ages of sampling whereas the seedlings under treatment showed a gradual change of the shoot apex from vegetative condition to reproductive panicle with each photoinductive cycle (Misra and Khan, 1970 a).

The short photoperiodic treatment, in general, greatly increased the sugar content of the leaves as compared with the control ones (Table I). In control sets the total and sucrose sugar content

TABLE I

Effect of photo-inductive cycles on sugar content (fresh weight) in a winter rice, BAM 3

Age of plants (days)	Control			No. of photo-inductive cycles	Treated		
	Total	Sugar % Reducing	Sucrose		Total	Sugar % Reducing	Sucrose
28	0.180	0.030	0.150	0	0.181	0.031	0.150
29	0.070	0.027	0.043	1	0.210	0.303	0.177
31	0.092	0.042	0.050	3	0.250	0.040	0.210
33	0.100	0.044	0.056	5	0.310	0.060	0.250
35	0.120	0.061	0.059	7	0.330	0.065	0.265
37	0.094	0.034	0.060	9	0.320	0.064	0.264
39	0.170	0.030	0.140	11	0.320	0.060	0.260

flowering in rice involves a respiratory shift was reported earlier (Misra and Khan, 1970). In the present experiment an attempt has been made to find out if the photoperiodic mechanism by which flowering is induced in rice has anything to do with age related shift in metabolites in the leaves, the organs of the reception of photoperiodic stimulus. The rice seedlings of a winter rice, BAM 3, raised in experimental pots were divided into 2 sets with 36 pots (144 plants) in each. Although the variety of rice used in this investigation crosses the basic vegetative phase or critical juvenile phase at the age of 14 days, it achieves complete development of the photoreceptive mechanism at the later age of 28 days (Misra and Khan, 1973). Therefore, at the age of 28 days, one set of seed-

lings showed more or less a gradual increase in quantity with an increase in the age of seedlings. The reducing sugar content showed an increase upto 35th day and then a decline thereafter. Photoinductive cycles remarkably increased the sugar content of the leaves as compared with that of control seedlings. The percentage of increase in sugar content of the leaves receiving seven consecutive cycles reached the maximum value compared to the rest of the treated seedlings. In the control seedlings the total, soluble and protein nitrogen content of the leaves showed a gradual quantitative increase with an advancement in the age of seedlings (Table II). The nitrogen content markedly increased with an increase in the number of photoinductive cycles

TABLE II

Effect of photo-inductive cycles on nitrogen content (dry weight) in a winter rice, BAM 3

Age of plants at sampling (days)	Control			No. of photo-inductive cycles	Treated		
	Total	N% Soluble	Protein		Total	N% Soluble	Protein
28	2.85	0.80	2.05	0	2.85	0.80	2.05
29	2.91	0.88	2.03	1	3.06	0.92	2.14
31	3.02	0.85	2.17	3	3.30	0.98	2.32
33	3.20	0.87	2.33	5	3.56	1.00	2.56
35	3.25	0.90	2.35	7	3.64	1.15	2.49
37	3.30	0.95	2.35	9	3.61	1.19	2.42
39	3.32	0.96	2.36	11	3.58	1.18	2.40

along with simultaneous advancement in the age of rice seedlings. The total, soluble and protein nitrogen levels reached the peak values under 7, 9 and 5 photoinductive cycles. Further increase in the number of photoinductive cycles resulted in a gradual decline in nitrogen content from the peak value. The data of the present investigation indicate that accumulation of more carbon and nitrogenous compounds would probably be necessary for ear initiation in rice plants.

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#### CHROMOSOME NUMBERS IN TERMITIDAE (ISOPTERA)

LITTLE is known about the chromosome numbers of termites. Stevens<sup>1</sup>, Benkert<sup>2-4</sup>, Stella<sup>5-8</sup>, Grassé<sup>9</sup>, Light<sup>10</sup> and Truckenbrodt<sup>11</sup> studied lower termites, and Banerjee<sup>12-13</sup> one higher termite.

Both sexes are known to be diploid and the sex-chromosome mechanism studied in lower termites seems to correspond to a ♂ XO : ♀ XX mechanism.

We studied higher African termites of the family Termitidae collected in Zaïre. Gonads from imagoes reared in the laboratory and from kings and queens were fixed in 1 : 3 acetic-alcohol and orcein-squashed.

We squashed the testes from males of 23 different Termitidae species and the ovaries from females of only 3 different *Cubitermes* spp. and 1 *Crenetermes* sp.

The testes of *Cubitermes exiguus* presented 20 chromosomes in metaphase I, 19 of which are spherical-shaped bivalents and 1 is a large ring which appears to be formed by a terminal pairing of two bivalents of approximately the same size. In metaphase II, 21 spherical-shaped chromosomes are present. In the ovaries all metaphases seen showed the haploid number  $n=21$ . Both sexes present the same diploid number of 42 chromosomes.

*Cubitermes exiguus* presents what we call the "normal" chromosome set-up of the Termitidae.

Here follows the list of chromosome numbers of the different Termitidae studied. After the species names, if known, the haploid number in male metaphase I ( $nI$ ) and in male metaphase II ( $nII$ ), the diploid number in male ( $2n♂$ ), the haploid number in female ( $n♀$ ), the diploid number in female ( $2n♀$ ) and ( $Im$ ) if observed on imagoes reared in the laboratory. Voucher specimens cited before the name and specimen are preserved in our laboratory.

*Termitidae*.—*Apicotermitinae* (sensu Sands<sup>14</sup>): (S 37, 38, 39) *Acidotermites praus* ( $nI:20$ ,  $nII:21$ ), (S 9, 10) *Microcerotermites* sp. 1, ( $nI:21$ ,  $nII:22$ ), (S 19) *Microcerotermites* sp. 2 ( $nI:20$ ,  $nII:21$ ), *Microcerotermites fuscolibialis* ( $nI:20$ ,  $nII:21$ ), (S 42, 43) *Microcerotermites* sp. 3 ( $nI:20$ ,  $nII:21$ ). *Termitinae* [(sensu Sands<sup>14</sup>)]: (S 1) *Cubitermes exiguus* ( $nI:20$ ,  $nII:21$ ,  $n♀:21$ ,  $2n♂:42$ ,  $2n♀:42$ ), (S 105) *Cubitermes weissi* ( $nI:20$ ,  $nII:21$ ,  $n♀:21$ ,  $2n♂:42$ ,  $2n♀:42$ ), (S 106) *Cubitermes sankurensis* ( $nI:20$ ,  $nII:21$ ,  $n♀:21$ ,  $2n♂:42$ ,  $2n♀:42$ ), (S 20, 21 22) *Cubitermes* sp. 1 ( $nI:20$ ,  $nII:21$ ), (S 87, 88) *Crenetermites* sp. 1 ( $nI:20$ ,  $nII:21$ ,  $n♀:21$ ,  $2n♀:42$ ), (S 33, 34, 35, 36) *Thoracotermites macrothorax* ( $nI:20$ ,  $nII:21$ ,  $2n♀:42$ ), (S 11, 12, 13) *Ophio-termites mandibularis* ( $nI:20$ ,  $nII:21$ ), (S 107) *Procutitermites* sp. 1 ( $nI:18$ ,  $nII:19$ ), (S 31, 32)

*Noditermes* sp. 1 (nI : 18, nII : 19), (S 5, 6, 7, 8)  
*Unguitermes bouilloni* (nI : 20, nII : 21, 2n♀ : 42),  
(S 27, 28 a) *Pericapritermes* sp. 1 (nI : 20, nII : 21),  
(S 70, 71) *Tuberculitermes bycanistes* (nII : 21).  
Macrotermitinae : (S 108) *Macrotermes* sp. 1 (nI :  
20, nII : 21, 2n♂ : 42), (S 109) *Pseudacanthotermes*  
sp. 1 (Im) (nI : 20, nII : 21), (S 110) *Odontotermes*  
sp. 1 (Im) (nI : 20, nII : 21), (S 112) *Protermes*  
sp. 1 (nI : 20, nII : 21, 2n♂ : 42). Nasutitermitinae :  
(S 23, 24) *Nasutitermes arborum* (nI : 20, nII : 21),  
(S 111) *Afrosbulitermes congoensis* (2n♀ : 42).

There is a chromosome constancy at the generic level as it appears from the list (see for ex. : genus *Cubitermes*), as Light<sup>10</sup> pointed out. Also a constancy at the family level with some exceptions ; either two chromosomes more, or four chromosomes less in mitotic divisions of the testes.

Seeing this constancy, a systematic and phylogenetic interest appears in the possibility of bringing together some genera presenting the same differences with regard to the "normal" number like the *Pro-cubitermes* and the *Noditermes*, and of splitting a genus not showing a constancy in chromosome numbers, like the *Microcerotermes*, where one studied species shows chromosome numbers differing from the "normal" number.

We studied the *Noditermes* more in detail and could establish that the different number in mitosis was the result of the fusion of 8 chromosomes two by two. This fusion seems to be stable and can also be observed in the different stages of meiosis.

The 42 diploid number in both sexes excludes the possibility of a male heterogametic sex corresponding to the XO arrangement in the studied species.

The ring (best seen in squash preparations) observed in male metaphase I of all the studied species reminds us, in some preparations, of the "parachute" type of sex-bivalent described by Stevens<sup>15-17</sup> ; but with two bivalents of approximately the same size.

The presence of this ring in male metaphase I suggests to us a heterogametic sex corresponding to the XY arrangement.

Did the higher termites develop their XY : XX arrangement from a "primitive" XO : XX mechanism ?

The colonies of *Cryptotermes* reared in the laboratory are still too young for the gonads to be squashed, so we have no results for lower termites.

The constancy in chromosome numbers in the two parent species of our crosses between two *Cubitermes* species and between *Ophiotermes mandibularis* and *Cubitermes weissi* (Bouillon and Vincke<sup>18</sup>) made the production of hybrids easier by giving the possibility of a complete pairing of the chromosomes. But nothing is known about the "fertility" of the descendants.

The author is indebted to Prof. Dr. A. Bouillon, Laboratoire d'Ecologie Animale, Université de Louvain, Belgium, to Prof. Dr. B. John, formerly of the Department of Zoology, University of Southampton, England, and to Prof. Dr. H. Van Den Berghe, Laboratorium voor menselijke genetica, Universiteit Leuven, Belgium, who let him work in their laboratories.

Dept. de Sciences de Base, PIERRE POL VINCKE.  
Faculté de médecine,  
Université Nationale du Zaïre,  
Campus de Kinshasa BP 118 Kin XI,  
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## SHORT SCIENTIFIC NOTES

### Rain Tree Fruit—A New Raw Material for Alcohol

Rain tree, *Albizia lebbek*, (Dirisana in Telugu), is a big tropical tree, largely grown for its shade. The fruit bears a remote resemblance to the fruit of the tamarind tree and consists of pods containing pulp and seeds. When unripe, the fruit is greenish, but when ripe, it becomes blackish and the pulp tastes sweet. The ripe fruit drops down and collects under the trees and is eaten by goats and cattle.

The ripe fruit has been found to contain 15% moisture, 17% reducing sugar and 38% total reducing sugar as glucose. The fruit (100 gm), crushed and fermented whole with addition of water and a pure culture of distillery yeast, gave a net yield of 20.5 cc of absolute alcohol, 82% of the theoretical yield. This yield corresponds to about 45 gallons of absolute alcohol per ton of the fruit. Water extract of the fruit has also been separately fermented and has more or less confirmed the yield of alcohol.

Rain tree fruit is worth exploiting for alcohol according to need and convenience. For fermentation and recovery of alcohol on large scale, the fruit would need to be crushed and extracted with water and strained.

Machilipatnam.

B. G. KRISHNAMURTI.

October 3, 1974.

### Occurrence of the Household Insect, *Asura conferta* Wlk. (Arctiidae: Lepidoptera) on Field Crops

*Asura* (= *Nepita*, *Pitane*) *conferta* Wlk.<sup>3</sup>, a household insect, distributed in sub-montane districts of southern India, was found feeding on moss and lichens<sup>2</sup>. The larva was not known to damage crops but formed a peculiar noxious pest owing to the large numbers in which it often occurred in houses and the irritating nature of its hairs<sup>1</sup>.

During July–November, 1973 and July–August, 1974 the hairy caterpillar of *A. conferta* was observed feeding on mulberry, brinjal, ragi and jowar leaves in Hebbal, in addition to feeding on moss and lichens, and invading the houses, especially the walls. The young caterpillars were feeding gregariously on the undersurface of the leaves, leaving only the upper epidermis. As a result the leaves were skeletonised and curled downward and dried. The grown up caterpillars defoliated the leaves, especially the tender ones, and migrated

from plant to plant by means of silken threads spun by them.

This insect was observed to damage the field crops for the first time, showing the possibility of its becoming a regular crop pest.

Dept. of Entomology, B. L. VISWESWARA GOWDA.  
Univ. of Agril. Sci., H. C. BHOGE GOWDA.  
Hebbal, B. N. VISWANATH.  
Bangalore 560024, August 30, 1974.

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### A Rapid Test for the Identification of Virus-Infected Groundnut (*Arachis hypogaea* L.)

The application of diagnostic tests for identifying the virus-infected plants has been found to be useful either in eliminating sources of virus inoculum in the field or obtaining virus-free seed materials. Such tests are based on differential colour reactions of infected tissues<sup>1</sup> or serological tests. The bud blight disease of groundnut is assuming importance in many districts of Tamil Nadu State because of its widespread occurrence. The development of technique based on which the infected plants can be eliminated at the earliest possible time would help to reduce the spread of the disease.

Various parts of the infected groundnut plants, viz., leaf, petiole, stem and root were tested. The petiole was found to be the suitable tissue for the test. Free-hand sections of petioles of the second leaves from the top of the healthy, infected plants and the branches of infected plants showing no apparent symptoms of the disease, were taken. The cross-sections were immersed in 0.05% phloroglucinol in alcohol for 5 minutes. Then the sections were transferred to concentrated hydrochloric acid in which they were placed for 30 to 60 seconds. The sections were then washed in distilled water and observed under the microscope. Fifty sections from petioles of healthy and infected leaves and the leaves from branches showing on apparent symptoms were examined.

In the petioles from the healthy plants, the xylem was faintly stained and appeared light pink in

colour. But the phloem of the petioles of infected leaves, in addition to xylem, showed positive reaction with the stain and were deeply stained. They appeared pinkish orange in colour. Similar reactions were observed in the sections from the petioles of leaves showing no apparent symptoms of infection. The absence of stain in the phloem tissue of healthy petioles clearly distinguished the healthy plants from infected plants.

Dept. of Plant Pathology, P. NARAYANASAMY,  
Tamil Nadu Agril. University, C. NATARAJAN,  
Coimbatore-3, May 31, 1974.

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#### An Additional Host for *Cercospora jasminicola* Mueller and Chupp.

The cultivated jasmynes, constituted by number of species of *Jasminum*, are affected by leaf spot and leaf blight caused by *Cercospora jasminicola*. During September–October, a severe leaf spot and leaf blight was observed on *Jasminum humile* Linn. at the Coimbatore campus of the Tamil Nadu Agricultural University.

The disease appeared on the upper surface of the leaflets as circular reddish brown to chocolate

brown spots 3 to 8 mm in diameter. In severe cases, the spots enlarged in size as irregular patches, covering the whole leaf surface. Brown to chocolate brown spots were also noticed on the petioles and stem.

Conidiophores are in dense fascicles, pale to olivaceous brown, unbranched, tips light coloured almost hyaline, septations few, and measure  $5-22 \mu \times 3-4 \mu$ . Conidia lightly olivaceous, cylindrical, septations indistinct, 3–5 septate and measure  $2-3 \mu \times 20-90 \mu$  (mean  $2.3 \times 4.5 \mu$ ). The pathogen was identified as *Cercospora jasminicola* Mueller and Chupp. *J. malabaricum* W., *J. sambac* Ait. from Dharwar, *J. rigidum* Zenk from Nandi Hills, *J. grandiflorum* L. from Coimbatore and *Jasminum* sp. have been so far reported as the hosts of *Cercospora jasminicola*. *J. humile* L. has not so far been reported as a host for this species and this is the first record.

Dept. of Plant Pathology, T. K. KANDASWAMY,  
Tamil Nadu Agril. Univ., C. L. SUBRAMANIAN,  
Coimbatore 641003,  
September 11, 1974.

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## REVIEWS AND NOTICES OF BOOKS

**The Use of Satellite Pictures in Weather Analysis and Forecasting**—Technical Note No. 124 (Revised Version of Technical Note No. 75). Edited by R. K. Anderson and N. F. Veltishchev. (WMO No. 333, Geneva, Switzerland). 1973. Pp. 275.

The launching of the first TIROS satellite in 1960 opened up a new era in the field of meteorology. Large number of satellites such as Nimbus, NOAA/ITOS and Meteor which have been launched since then, and the rapid advances made in the interpretation of satellite cloud pictures now provide a very valuable tool for more accurate weather prediction. The data from APT installations at various locations are now being used by a large number of countries for weather prediction. At the same time, a great need has been felt by a large number of meteorological observers for a hand book giving guidance for the interpretation of the data from these satellites. The present book *The Use of Satellite Pictures in Weather Analysis and Forecasting*, which is an updated version of

the earlier World Meteorological Organization Technical Note No. 75, provides a valuable up-to-date guide to all professional meteorological observers.

The Technical Note has been divided into 5 chapters. Chapter 1 gives an elementary description of the general characteristics of the two types of images that are currently available from weather satellites, namely, visible data obtained by television cameras and infrared data obtained by scanning radiometers. This chapter also briefly touches upon the limitations in the available resolution of instrumentation that are currently being used on various satellites.

Chapters 2, 3 and 4 provide an extremely good guidance for the interpretation of satellite pictures with a large number of illustrations of various cloud types, cloud patterns ranging from mesoscale to planetary scale. Examples of litho and hydro-meteors observable by satellites and distinctive features of earth surfaces including the quantitative evaluation of variation of snow and ice both on

land and sea surfaces, which are very important for proper interpretation for cloud formation, are also discussed in this chapter.

Chapter 3 describes the use of satellite pictures for locating different pressure patterns in the upper-air such as cyclones, anticyclones, troughs and ridges. A detailed description of tropical storms with appropriate illustrations are particularly of great value to the meteorology of tropics where the presently available data through conventional methods are limited.

In the last chapter, methods are described for estimating the wind direction and speed, relative humidity and amount of precipitation and turbulence from cloud images.

With the large number of illustrations the Technical Note carries, this Note is extremely useful guide to all those who are involved in Meteorological Forecasting. Other meteorologists who are interested in the development of sensors particularly for satellite application will also find this guide immensely valuable.

U. R. RAO.

**Physics, with Illustrative Examples from Medicine and Biology.** Vol. 1: *Mechanics*. By George B. Benedek and Felix M. H. Villars. (Addison-Wesley Publishing Company, Massachusetts 01867), 1973. Pp. 684. Price not given.

During the last few decades, chemistry has been increasingly used in the understanding of the behaviour of living systems, and biochemistry has flourished as an interdisciplinary area. However, increasingly it has been felt that biology and medicine require the application of the physical sciences for a deeper understanding of important problems and for solving these. Therefore, students and workers in the life sciences find it necessary to have a thorough grasp of the physical sciences.

The series under review has been brought out to fill this need and the first book, Vol. 1, deals with the principles of mechanics, taking illustrations wherever possible from biology and medicine. Although the treatment is elementary, all the ideas are carried through to their logical conclusion and the treatment throughout is thorough, even making use of differential equations, which are fundamental to mechanics. The book would, therefore, be found to be extremely valuable by persons who wish to give a course in Physics for students of biology and medicine. The following chapter

headings: 1. Kinematics; 2. Dynamics; 3. Static Equilibrium; 4. Momentum; 5. Work and Energy; 6. Feedback, Control and Stability in Physical and Biological Systems, give an idea of the contents, but the book is really very much more thorough and detailed than what can be illustrated in mere chapter headings. For instance, under Work and Energy, section 5.4 deals with the First Law of Thermodynamics in which the conservation of mechanical energy, external work and heat is considered in good detail. Under the same subsection, there is another discussion dealing with animal metabolism, work and the first law of thermodynamics. Similarly, in chapter 6 dealing with Feedback and other subjects, we find section 6.2 dealing with a typical mechanical system under automatic control—namely, the Steam Engine and Its Centrifugal Governor. Similarly, Temperature Control Using Feedback is discussed in appreciable detail and this is applied to study of control of body temperatures and a similar phenomenon, namely, control of blood glucose level.

The above, necessarily brief, information would give a clear idea of the novel approach to the learning of physics from biology and practical examples which the authors have adopted in the book under review. The book is brought out as a paper-back, but in a substantial size of  $8\frac{1}{2}/11"$ . It is understood that a separate printing has been made in Philippines by the Publishing Company, and no doubt this may be available in India in a cheaper version. The Reviewer has no hesitation to recommend this book to every laboratory in biology and medicine and even to laboratories in physics. At present, attempts are being made in India to evolve courses of study in biophysics and the more elementary portions of this course could readily adopt the style and method of presentation of physics, as adopted by the authors of this beautiful volume.

G. N. RAMACHANDRAN.

#### ERRATA

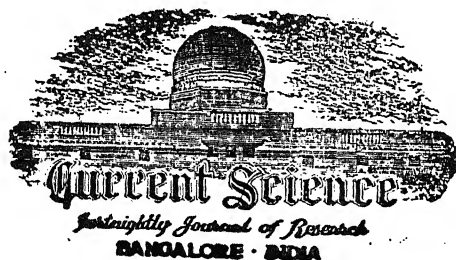
*Current Science*, Vol. 43, No. 19, October 5, 1974.

Page 611, last para, second line—insert 'cubes' for 'tubes'.

Last line—comma in place of full stop after the word 'Family'.

Page 612, second paragraph, second line—208--9° for 278--9°.

Page 612, fourth paragraph, third line— $\text{CHCl}_3$ :  $\text{MeOH}$  for  $\text{CHCl}_3$ ,  $\text{MeOH}$ .



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## GEL COUNTING OF $^{14}\text{C}$ AND $^3\text{H}^*$

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### ABSTRACT

Aluminum stearate gel in toluene base scintillator is employed for the measurement of varied amounts of carbon-14 labelled  $\text{BaCO}_3$  and tritiated lysine and the counting efficiencies are compared with the solution counting.  $\text{BaCO}_3$  particulates in the ranges of  $74 < X < 105 \mu$ , dia. and  $105 < X < 416 \mu$  dia. do not affect the gel counting efficiency. Sample weights upto 100 mgm do not cause any significant change in the gel counting efficiency while further increase in weight definitely lowers the efficiency. A comparison is made of the direct counting of carbon-14 labelled  $\text{BaCO}_3$  particulates with that of the gel suspension counting. Gel counting efficiency for tritium is found to be of the order of 1% of the solution counting. For carbon-14 this is about  $92 \pm 4\%$  of the solution counting in the weight range upto 100 mg  $\text{BaCO}_3$ .

### INTRODUCTION

**L**IQUID scintillation counting technique is the most common method in the recent years for the measurement of soft beta activity. In fact, any radionuclide that can be incorporated into or suspended in a scintillator solution, can be counted with a liquid scintillator system. The scope of liquid scintillation technique is enhanced with the advent of numerous heterogeneous systems<sup>1-8</sup> wherein the problems of solubility or quenching are significantly eliminated.

In the present investigations, a suspension technique using aluminum stearate gel in toluene, PPO and POPOP scintillator system is tried for the measurement of  $^{14}\text{C}$  and  $^3\text{H}$  in solid samples and the counting efficiencies relative to solution counting are obtained. For this purpose,  $^{14}\text{C}$  labelled  $\text{BaCO}_3$  and tritiated lysine are used. A comparison is made of the direct counting of  $\text{Ba}^{14}\text{CO}_3$  in toluene scintillator with that of the gel suspension technique.

### MATERIALS AND METHODS

**Gel preparation.**—Toluene scintillator was prepared with 6.5 gm PPO and 0.13 gm POPOP in one litre distilled toluene. To 10 ml of this scintillator, 1.5 gm aluminum stearate was added, mixed thoroughly and heated in a water-bath at  $80^\circ\text{C}$  for 5 minutes<sup>1</sup>. The gel formed was uniform and even after stirring with the suspended particulates, attains uniformity immediately without air bubbles. Similar gel prepared with dioxan scintillator (6.5 gm PPO, 0.13 gm POPOP, 100 gm naphthalene in one litre distilled dioxan) showed less uniformity and was unstable after shaking; hence the former was used. The active material was incorporated into the toluene scintillator solution prior to gelling and this resulted in a fairly uniform distribution of the sample in the gel.

**$^{14}\text{C}$  labelled  $\text{BaCO}_3$ .**— $^{14}\text{C}$  was obtained from the Isotope Division, BARC, in the form of  $\text{Na}_2^{14}\text{CO}_3$  (8 uci/110  $\mu\text{gm}$   $\text{Na}_2^{14}\text{CO}_3$ ). A stock solution was prepared from this, containing  $4.4 \times 10^4$  dpm/ml. A definite amount of this activity was added to known amounts of inactive  $\text{Na}_2^{14}\text{CO}_3$  (10 mg/ml) and mixed thoroughly. Excess  $\text{BaCl}_2$  solution was added to precipitate  $\text{BaCO}_3$ . The precipitate was coagulated by heating in water-bath at  $90^\circ\text{C}$  for 5 minutes, centrifuged and washed with distilled water thoroughly to remove the excess  $\text{BaCl}_2$  reagent. The precipitate was then dried under infrared lamp, powdered, sieved and used for the experiments.

**Gel counting of  $\text{Ba}^{14}\text{CO}_3$ .**—A bulk amount of labelled  $\text{Ba}^{14}\text{CO}_3$  was prepared using known amounts of inactive sodium carbonate and  $^{14}\text{C}$  activity. The  $\text{BaCO}_3$  precipitate was dried, powdered and sieved through Greenings Test meshes 25 and 36 (Middlesex U.K.), to get the particle sizes in the range of  $416 < X < 675 \mu$  dia. Varied amounts (20–370) mgm of these particulates were suspended in toluene-aluminum stearate scintillator gel and counted to study the effect of weight in gel counting efficiency (Fig. 1). An aliquot of the added  $^{14}\text{C}$  activity was measured using dioxan scintillator for comparison (solution counting).

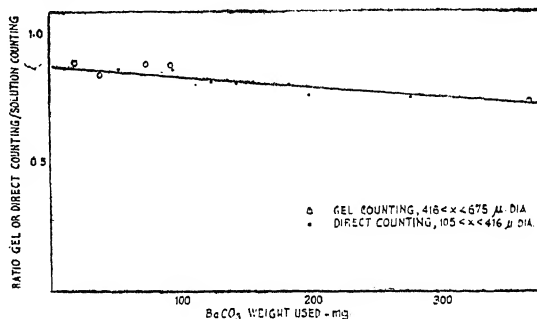


Fig. 1. Heterogeneous counting efficiency vs. weight.

\* Work carried out under research contract No. 1297/RB of International Atomic Energy Agency, Vienna.

In order to study the effect of change in the specific activity of  $\text{Ba}^{14}\text{CO}_3$  in gel counting efficiency, the following experiments were conducted: A set of 5 ml  $\text{Na}_2\text{CO}_3$  solutions, containing 50 mgm  $\text{Na}_2\text{CO}_3$  each ( $\sim 90$  mgm  $\text{BaCO}_3$ ), were spiked with different amounts of  $^{14}\text{C}$  activity and labelled  $\text{BaCO}_3$  was prepared as described earlier. The  $\text{Ba}^{14}\text{CO}_3$  particulates thus prepared were sieved to obtain particle size of  $74 < x < 105 \mu$  dia. and  $105 < x < 416 \mu$  dia. The particles were suspended in the stearate gel, counted and compared with the solution counting of the added  $^{14}\text{C}$  activity. A similar experiment was performed with the particle size of  $416 < x < 675 \mu$  dia. using 370 mgm of  $\text{BaCO}_3$  (Fig. 2).

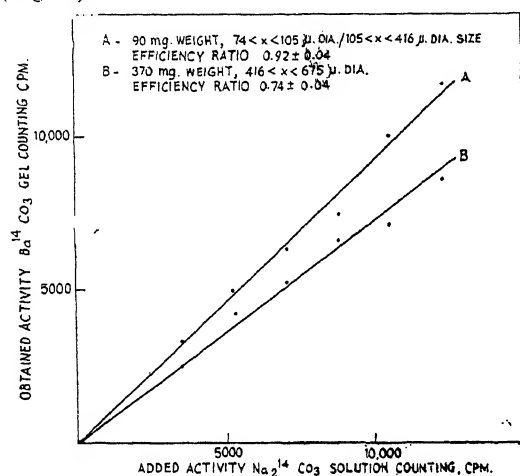


FIG. 2. Gel Counting Efficiency for varied specific activity of  $\text{Ba}^{14}\text{CO}_3$ .

**Direct counting of  $\text{Ba}^{14}\text{CO}_3$ .**—A study was made to determine the direct counting efficiency for  $\text{Ba}^{14}\text{CO}_3$  particulate suspensions in toluene scintillator system. Varied amounts of  $\text{Ba}^{14}\text{CO}_3$ , with particle sizes of the order of  $105 < x < 416 \mu$  dia. were used. The counting efficiency was computed relative to the counts obtained for aqueous  $^{14}\text{C}$  activity in dioxan scintillator (Fig. 1). Table I

TABLE I

Direct counting of  $\text{Ba}^{14}\text{CO}_3$  in toluene scintillator

BaCO <sub>3</sub> mg	Ratio of BaCO <sub>3</sub>	Solid counting w.r.t.		Solution counting
		particle size in $\mu$ dia.		
	74< $\times < 105$	105< $\times < 416$	416< $\times < 675$	675< $\times < 1000$
20	0.93	..	0.85	0.78
40	0.93	0.87	0.85	0.80
100	..	0.88	0.86	0.81
150	..	0.82	..	..
200	..	0.77	..	..
280	..	0.76	..	..

gives the direct counting efficiency for different amounts of labelled  $\text{BaCO}_3$  of varying particle sizes.

**Gel counting of  $^3\text{H}$ .**—Tritiated toluene obtained from the Isotope Division, BARC, was diluted with toluene to give 1100 dpm per ml. 10 ml of toluene scintillator was spiked with 1–5 ml of tritiated toluene. These were initially liquid counted. Different amounts of aluminum stearate (1–2 g) were added to these solutions, and gels prepared, and counted to study the effect of stearate gel on the count rate. The counting losses obtained might be either due to the absorption of the soft betas in the gel or due to the quenching of aluminum stearate. Quenching due to aluminum stearate was studied using 10 ml spiked scintillator and varied amounts of aluminum stearate (0.3–2.1 g) (Fig. 3).

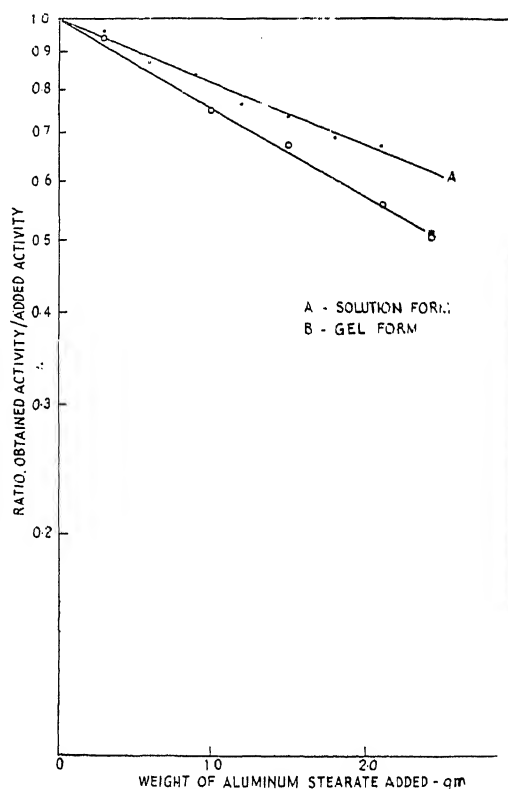


FIG. 3. Quenching due to Aluminum stearate in solution and gel form for tritium.

In order to study the effect of solid suspension of tritiated samples in the gel, 1 mc tritiated lysine with a specific activity of 1200 mc per millimole was diluted and a known volume was mixed with 200 mg of inactive lysine. The labelled lysine was recrystallised from ethanol medium, vacuum dried and weighed. A known portion of the solid was dissolved in water to give 1 mg per ml concentra-

tion. This was counted in dioxan scintillator to give the added amino acid activity (solution counting). Different amounts of labelled lysine (12–66 mgm) were suspended in toluene-stearate gel and counted. The ratio between the two would indicate the gel counting efficiency for tritium compared to the solution counting (Table II).

TABLE II

*Gel counting efficiency of tritiated lysine relative to the solution counting*

Weight of amino-acid added mg	Activity of the amino-acid		Ratio of gel count to solution count
	Solution count cpm	Gel count cpm	
12.1	11080	141	0.0127
25.0	22880	250	0.0109
39.6	36230	391	0.0108
66.5	60850	640	0.0105

#### RESULTS AND DISCUSSION

The weight vs efficiency relation by the gel counting or direct counting technique is shown in Fig. 1. Increased sample weights decrease counting efficiency. Even as large samples as 369 mg could be counted with an efficiency of 0.74 (ratio) of that of solution counting. The direct counting follows the same trend. Nathan *et al.*<sup>9</sup> have reported a loss of less than 25% for five fold weight increase using thixcin-toluene system in 200–1000 mgm range.

Figure 2 shows the linearity of the gel counting efficiency for the varied specific activity of  $\text{Ba } ^{14}\text{CO}_3$ . For particle sizes of  $74 < \times < 105 \mu$  dia. and  $105 < \times < 416 \mu$  dia., the efficiency is found to be 0.92 when 90 mgm sample used. The same has decreased to 0.74 for the particles in the range of  $416 < \times < 675 \mu$  dia. for a sample weight of 369 mg. This may either be due to the increased sample weight or due to the increased particle size. However, from the present studies it can be concluded that particles upto the sizes of  $105 < \times < 416 \mu$  dia. will not significantly affect the counting efficiency. White and Helf<sup>2</sup> have reported that once particle size is reduced to less than 60 mesh, further reduction in size does not affect the counting efficiency, though many others<sup>3,9</sup> feel that sieving of the  $\text{Ba } ^{14}\text{CO}_3$  prior to incorporation into gel as unnecessary.

The relatively large counting efficiency obtained for direct particulate suspensions could be used advantageously. Hayes *et al.*<sup>3</sup> reported the first evidence that liquid scintillation method could be successfully used for the measurement of materials in suspension, rather than in solution. In their studies,  $\text{Ba } ^{14}\text{CO}_3$  was finely ground, moistening with ethanol before incorporation into the toluene scintillator which seems to be unnecessary from the present studies. Larger particle sizes, even after settling to the bottom, could be counted

without much loss in efficiency. Table I shows the efficiency ratios of direct counting of varied particle sizes of  $\text{Ba } ^{14}\text{CO}_3$ . There is very little difference in the efficiency for large particle sizes ( $675 < \times < 1000 \mu$  dia.) and the finest particle sizes employed ( $74 < \times < 105 \mu$  dia.) since the scintillator solution diffuses through the pores of the particles of  $\text{Ba } ^{14}\text{CO}_3$  and  $^{14}\text{C}$  betas do not see much of the absorbing material before they produce photons. The good results of the method make it attractive at least for the measurement of high count rates.

The aqueous counting of  $\text{Na}_2^{14}\text{CO}_3$  with sufficient inactive carrier has some disadvantages. When  $8 \times 10^{-3} \mu\text{Ci}$  containing 1 mg  $\text{Na}_2\text{CO}_3$  was counted in 10 ml scintillator, the activity gradually decreased and attained a stable value very slowly. Heterogeneous system of counting, viz., suspension with gel, is well suited even in such cases where the specific activity of the sample is less, since large amounts can be used without giving rise to this phosphorescence effects.

Quenching due to aluminum stearate in solution and in the gel form for tritiated toluene is given in Fig. 3. The nett efficiency obtained for  $^3\text{H}$ , when 1.5 g aluminum stearate gel is formed in 10 ml toluene base scintillator, is 0.67 times that of solution counting. The self-absorption loss of tritium soft betas (18 kev) is so predominant even with lysine powder that it could be counted by suspension technique only with very small efficiency, 1% of the solution counting. Since the solution counting efficiency for tritium itself is 25%, the absolute counting efficiency for suspension technique will be of the order of 0.25%. Wang<sup>10</sup> also obtained counting efficiency for tritium as low as 1% when a paper strip containing absorbed tritium activity was dipped directly in a scintillator solution. The gel counting efficiency is low for tritium, but the linearity with respect to the total activity is well maintained. Thus gel counting for tritium is found to be not as attractive as that for carbon-14.

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## 2, 3, 5-TRIPHENYLTETRAZOLIUM CHLORIDE AS A NEW REAGENT FOR THE DETECTION OF SULPHIDE AND SOME ORGANIC THIOCOMPOUNDS

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### INTRODUCTION

FEIGL<sup>1,2</sup> described the detection of submicrogram quantities of organic sulphur compounds based on their catalytic action on the reaction between iodine and azide. The detection of sulphide was affected by using sodium nitroprusside<sup>3</sup>, dimethylaniline, *p*-phenylene diamine<sup>4</sup> as chromogenic reagents. Several chromatographic<sup>5,6</sup> and crystallographic<sup>7</sup> methods also had been reported. A survey of the literature shows very few<sup>8,9</sup> reports for the detection of sulphur in organic compounds. Recently, Feigl critically reviewed the reagents for the detection of other sulphur compounds<sup>10</sup>. Sodium plumbite was used for the detection of thioacetamide. Phosphomolybdic acid, *p*-nitrosodimethyl aniline, pentacyanoammine ferroate and 2,6-dichloroquinone-4-chloramine are the reagents used for thiosemicarbazide. Raney alloy, sodium nitroprusside, phosphomolybdic acid, selenous acid are used for dithioamide. Most of the methods described using the reagents mentioned, for the detection of sulphur compounds are time consuming and involve heating on steam bath. These methods are susceptible to the interference of thiourea, thioglycolic acid, semicarbazide, urea and hydroxylamine. In this communication we report the use of 2,3,5-triphenyltetrazolium chloride for the detection of the sulphide, thiosemicarbazide, dithioamide and thioacetamide in weakly alkaline medium. The new method involves the extraction technique and has the advantage that it can be carried out in the presence of urea, thiourea, semicarbazide, Hydroxylamine thioglycolic acid and anions like chloride, bromide, iodide, acetate, oxalate and citrate.

### EXPERIMENTAL

**Reagents.**—0.1% solution of 2,3,5-triphenyl tetrazolium chloride was prepared by dissolving G.R., E. merck grade sample in deionized water. The solution must be stored in a dark place as its exposure<sup>11,12</sup> even to diffused light, on long standing will result in the formation of the coloured product.

0.1% solution of sodium sulphide, thioacetamide, thiosemicarbazide and dithioamide were prepared by dissolving Analar, B.D.H. samples in deionized

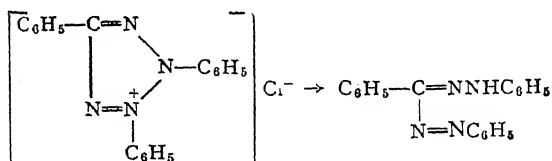
B.D.H. Analar quality methyl isobutyl ketone was used without further purification.

### PROCEDURE

0.1 ml test solution is mixed with 0.2–0.4 ml of sodium hydroxide (0.02 M) and 0.2 ml of 2,3,5-triphenyl tetrazolium chloride (0.1%) in a semi-micro test tube, and the volume was made to 1 ml with deionized water. The mixture is equilibrated with 0.2 ml of methyl isobutylketone for 1–2 minutes. The appearance of the reddish brown colour in the extract after equilibration shows the presence of the sulphur compounds.

### RESULTS AND DISCUSSION

Table I gives the identification and dilution limits of various sulphur compounds. Under the experimental conditions the reagents do not give any colour in the organic phase even after 10 hours at 35° C in the absence of sulphur compounds. In alkaline medium, the sulphur compounds other than sulphide may hydrolyse resulting in the formation of sulphide, which in turn reacts with the reagent resulting in the reddish brown coloration in the extract. The extract was found to be triphenyl formazan<sup>13,14</sup> as its spectrum exhibits maximum absorption band in the region of 480–490 nm.



2, 3, 5-Triphenyl  
Tetrazolium Chloride

Triphenyl Formazan

TABLE I  
Identification and dilution limits

Sulphur compound	Identification limit μg in 1 ml	Dilution limit
Sodium sulphide ..	1.0	1 : 1 × 10 <sup>5</sup>
Thioacetamide ..	10.0	1 : 1 × 10 <sup>4</sup>
Thiosemicarbazide ..	2.0	1 : 5 × 10 <sup>4</sup>
Dithio oxamide ..	5.0	1 : 2 × 10 <sup>4</sup>

### INTERFERENCES

The amounts of ions and substances indicated did not effect the test.

5000 ppm of  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ , oxalate, citrate, sulphite, phosphate, nitrate, nitrite, tartrate, carbonate azide, acetate, bromate and 1000 ppm of urea, hydroxylamine, semicarbazide, thiourea, glucose, starch, 50 ppm of thioglycollic acid do not interfere, while even minute quantities of thiocyanate, perchlorate, periodate and iodate obscure the colour formation.

#### ACKNOWLEDGEMENT

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### OCCURRENCE OF AFLATOXINS AND CITRININ IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) AT HARVEST IN RELATION TO POD CONDITION AND KERNEL MOISTURE CONTENT

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#### ABSTRACT

Groundnut pods were collected from fields on the day of harvest in November, 1972, graded into undamaged and damaged pods and kernel moisture content was determined. The accumulation of a yellow pigment in some groundnut kernels, especially in damaged pods, was noticed and it was identified as citrinin. Only *Aspergillus flavus* isolates were found to produce aflatoxins while isolates of *Penicillium citrinum*, *P. jensenii* and *A. terreus* produced citrinin. High levels of aflatoxins and citrinin were associated with kernels having less than 30% of moisture, which occurred under rain-fed conditions. Where the moisture content of kernels is high (under irrigation) there was very little or no formation of the mycotoxins. In all the cases damaged kernels were found to contain the toxins. Kernel moisture content and pod damage appear to be the major governing factors for fungal infestation and toxin accumulation before harvest.

#### INTRODUCTION

**P**RESENCE of aflatoxins in groundnut kernels before harvest<sup>8</sup> and at the time of harvest<sup>6-14</sup> had been reported. Their presence was attributed to low kernel moisture content (30%), over maturity, unfavourable weather conditions and excessive pod damage. *Aspergillus flavus* Link. invades groundnut pods in the field before harvest, during storage or during handling<sup>5,6,13,15</sup>. Optimum kernel moisture content of 20-30%<sup>2</sup> and temperature of 25°-35°C<sup>9</sup> are favourable for aflatoxin production. Kernels are more susceptible to fungal infestation and toxin production when their moisture content is between 10 and 30%<sup>8</sup>.

During our studies on fungal infection of groundnut and aflatoxin accumulation before harvest, we found an accumulation of a yellow pigment in groundnut kernels, especially in kernels from rotted pods (Fig. 1). Such kernels emit golden yellow fluorescence when exposed to UV light (Fig. 2). The chemical nature of this substance was determined and the conditions for its accumulation was also investigated.

#### MATERIALS AND METHODS

Groundnut samples were collected from the fields around Tirupati (A.P.), on the day of harvest in November, 1972 from three different localities. Two samples of 1 kg of pods for each were collected in polythene bags. The pods were graded into undamaged (sound mature) and damaged pods

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(rotted, insect bored, broken shells, etc.). The moisture content to kernels was determined by drying 20 g of sliced kernels for 5 hr at 102° C, cooled in a desiccator and weighed.



FIG. 1. Damaged pods of groundnut.

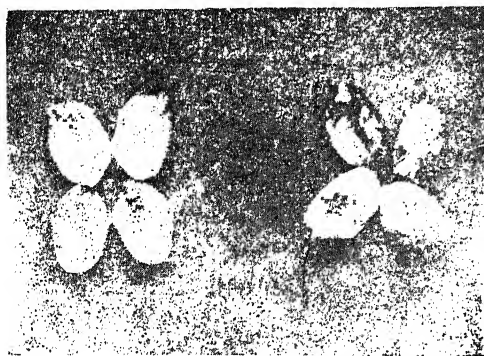


FIG. 2. Undamaged and damaged groundnut kernels as seen under Ultraviolet light.

From each sample 100 kernels were surface sterilized by immersing for two minutes in 0.1%  $\text{HgCl}_2$ , rinsed repeatedly in sterilized distilled water and plated out on Czapek-Dox rose bengal streptomycin agar (CDRSA) in petridishes (5 seeds/9 cm plate). The plates were incubated at  $28 \pm 2^\circ \text{C}$  and examined for fungi after 4 and 8 days. Fungi coming out from the kernels were counted and isolated on potato dextrose agar (PDA) slants. The isolates were purified by single spore isolation in case of sporulating fungi or by cutting and subculturing the hyphal tips in the case of non-sporulating fungi.

Groundnut samples were analysed for mycotoxins. 50 g of each sample was extracted with 250 ml of methanol-water (55; 45, V/V) plus 100 ml of *n*-Hexane in a waring blender. After centrifugation of the mixture, 50 ml of the aqueous methanol layer was shaken with two 50 ml portions of chloroform. The combined chloroform extracts

were evaporated on water bath, residue was redissolved in 1 ml of chloroform and analysed for aflatoxins by thin layer chromatography (TLC). Another 50 ml of the aqueous methanol layer was acidified to 1.5 to 2.0 pH with conc. sulphuric acid and extracted as above and was analysed by TLC<sup>11</sup>. The following solvent systems were used: Toluene; Ethyl acetate; 90% Formic acid (60; 30; 10, V/V/V) and Ether; Methanol; Water; 90% Formic acid (95; 4; 1; 1, V/V/V/V) in a tank lined with filter paper. The plates were viewed under UV light and estimated quantitatively by comparing the intensity of fluorescence of spots from extracts with that of standards. The kernels which showed the yellow pigment were found to contain citrinin by this method.

The fungal isolates were screened for aflatoxin and citrinin production by the method of van Walbeed *et al.* (1968). They were grown on PDA slants enriched with 0.2% yeast extract. Conidial or mycelial suspension was made with sterile water containing Tween-80 (0.05%) and 0.1 ml of this suspension was added to a test-tube containing 5 ml of yeast extract sucrose (YES) medium (15 g of sucrose, 2 g of yeast extract and 100 ml of distilled water). The test-tubes were incubated in a slanting position for one week at  $28 \pm 2^\circ \text{C}$ . For each isolate 5 replicates were maintained. The culture filtrate of all the replicates pooled and extracted by shaking vigorously for two minutes with 50 ml of hot (60° C) chloroform in a separating funnel. The lower chloroform layer was passed through anhydrous sodium sulphate in a column. The column was washed with another 10 ml of hot chloroform and the washings were pooled, evaporated on water bath to dryness and kept in deep freeze (0° C) for TLC analysis.

The extracts were redissolved in 0.5 ml of chloroform and appropriate amounts were spotted on TLC plates along with authentic samples of aflatoxins and citrinin. The following solvent systems were employed to detect the toxins in the extracts. Chloroform; Acetone (9; 1, V/V). Chloroform; Methanol (93; 7, V/V), Toluene; Ethyl acetate; 90% Formic acid (60; 30; 10, V/V/V) and Benzene; Methanol; Acetic acid (24; 2; 1, V/V/V). Extracts containing fluorescent materials having the colour and  $R_f$  values similar to those of authentic aflatoxins and citrinin were co-chromatographed.

#### RESULTS AND DISCUSSION

The infestation of kernels and the accumulation of aflatoxins and citrinin in kernels obtained from undamaged and damaged pods were estimated in 1972 Kharif crop (August–November) under rainfed conditions and under supplement irrigation

TABLE I  
Fungal infestation and toxin content in groundnut kernels in 1972-Kharif Crop

Water source	Pod condition	% of Kernels infested with			% of clean Kernels	Kernel moisture content %	Toxin content (µg/kg)	
		<i>A. flavus</i>	<i>Penicillium</i> sps.	Other fungi			Aflatoxins	Citrinin
Rain-fed	Undamaged	32	76	82	18	29.2	940	10
Irrigated	„	25	50	55	45	46.0	68	0
Rain-fed	Damaged	85	100	100	0	35.0	4980	950
Irrigated	„	100	100	100	0	50.1	2100	86

TABLE II  
Toxin content and kernel moisture content in groundnut at harvest in relation to pod condition and nature of water source

Crop season	Water source	Pod condition	No. of samples tested	Range of moisture content (%)	No. of samples containing				Range of aflatoxins content (µg/kg)	Range of citrinin content (µg/kg)
					Only aflatoxins	Only citrinin	Aflatoxins + citrinin	No. toxins		
1972 Kharif	RF	UDP	25	28-31	12	0	5	8	360-1200	0-60
	I	UDP	20	38-46	6	0	0	14	Tr-130	0
	RF	DP	25	25-31	20	0	5	0	2500-5850	200-140
	I	DP	20	45-40	17	0	3	0	1500-2250	Tr-1200
1973 Rabi	I	UDP	10	42-45	0	0	0	10	0	0
	I	DP	10	41-43	8	0	0	2	Tr-210	0
1973 Kharif	RF	UDP	15	28-32	6	0	1	8	176-950	0-Tr
	I	UDP	10	40-42	1	0	0	9	0-Tr	0
	RF	DP	15	30-33	13	0	2	0	750-2800	70-150
	I	DP	10	40-44	10	0	0	0	420-1850	0

RF=Rain fed; I=Irrigated; UDP=Undamaged pods; DP=Damaged pods.

separately (Table I). Kernel moisture content was lower while the percentage of kernels infested with fungi was much higher under rain-fed conditions than under irrigation. All the damaged kernels were infested with fungi.

Aflatoxin and citrinin content of kernels of undamaged and damaged pods collected from fields in 1973-Rabi (February-May) and 1973-Kharif were also estimated (Table II). No toxins were found in 1973-Rabi samples and only a low per cent of samples containing toxins were found in 1972 and 1973 Kharif under irrigated conditions. The range of aflatoxins and per cent of samples containing toxins was high in samples of rain-fed plots in 1972 and 1973 Kharif. In all the cases damaged kernels were found to contain the toxins but the toxin content was much higher under rain-fed conditions.

In all the cases the presence of aflatoxins and citrinin were confirmed by spraying *p*-anisaldehyde<sup>10</sup>. The presence of aflatoxins and citrinin was further confirmed by bioassay using *Bacillus megaterium* (NRRL 1368), *B. brevis* (NRRL 1874) and *B. subtilis*. The spots on TLC plates were eluted and dissolved in chloroform, centrifuged and the chloroform layer was evaporated to dryness. They were redissolved in chloroform. These eluates were transferred to Whatman No. 1 filter paper discs (3 cm), dried and placed on seeded agar plates. The inhibition zones were noticed after 24 hr.

Out of 233 isolates belonging to 19 species of fungi tested for aflatoxins and citrinin production, only *A. flavus* isolates (38 out of 52) produced aflatoxins. *A. terreus* (5 out of 14) and all the isolates of *Penicillium citrinum* and *P. jensenii*

produced citrinin (Table III). Out of 38 aflatoxin producing *A. flavus* isolates, 29 produced  $B_1 + G_1$  and the others produced  $B_1 + G_1 + B_2 + G_2$ .

Kernel moisture content and pod damage appear to be the major governing factors for fungal infestation and toxin accumulation before harvest. Hundred per cent kernel infestation was observed in damaged pods and this is said to be due to the exposure of kernel surface to the soil<sup>1,3,4,7,12</sup>. Even in undamaged pods high per cent of fungal infestation was observed in rain-fed plots. Here the kernel moisture content seems to exert much influence on fungal infestation because kernel moisture was around the maximum limit of 30% for aflatoxin accumulation. Though the per cent of kernels infested by fungi in damaged pods in both

field. Even in the damaged pods toxin accumulation seems to be governed to a greater extent by kernel moisture content. From this point of view supplement irrigation of fields during *Kharif* season is beneficial. Another interesting aspect brought out by this study is the finding that citrinin also accumulates in groundnut kernels in the field, which has not been so far reported.

#### ACKNOWLEDGEMENTS

We are very grateful to Dr. P. M. Scott, Food Research Division, Department of Health and Welfare, Ottawa, Ontario, Canada, for a gift sample of citrinin and also for his suggestions during this investigation; Dr. C. W. Hesseltine, Chief of fermentation laboratory, NRRL, Peoria, Illinois, for kindly supplying the bacterial cultures for bioassay; Dr. D. I. Fennell, NRRL, Peoria, Illinois and Dr. W. Gams, Centralbureau Voor Schimmelcultures, Baarn, The Netherlands for their help in identifying some of the fungal cultures.

TABLE III  
Aflatoxin and citrinin production by fungi isolated from groundnut kernels in 1972 Kharif crop.

Fungus	No. of isolates screened	No. of isolates producing aflatoxins	No. of isolates producing citrinin
<i>Alternaria tenuis</i>	2	0	0
<i>Aspergillus flavus</i>	52	38	0
<i>A. fumigatus</i>	12	0	0
<i>A. niger</i>	28	0	0
<i>A. terreus</i>	14	0	0
<i>A. ustus</i>	5	0	0
<i>Cunninghamella</i> sp.	1	0	0
<i>Fusarium equiseti</i>	2	0	0
<i>F. oxysporum</i>	12	0	0
<i>F. solani</i>	15	0	0
<i>Macrophomina phaseoli</i>	12	0	0
<i>Neochosmospora vasinfecta</i>	2	0	0
<i>Penicillium citrinum</i>	26	0	26
<i>P. funiculosum</i>	5	0	0
<i>P. jenseni</i>	20	0	20
<i>Penicillium</i> sp.	18	0	0
<i>Rhizoctonia solani</i>	1	0	0
<i>Rhizopus</i> sp.	5	0	0
<i>Sclerotium rolfsii</i>	1	0	0

irrigated and rain-fed plots is more or less same, higher levels of toxin accumulation were observed under rain-fed conditions. This is most probably due to low kernel moisture content in rain-fed plots.

This study indicates that fungal infestation as well as the kernel moisture content are important in determining the accumulation of toxins in the

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## LETTERS TO THE EDITOR

### SPECTROGRAPHIC DETERMINATION OF LEAD AND TELLURIUM IN Bi-Pb AND Bi-Te ALLOYS

BISMUTH metal, when alloyed with lead and tellurium, is useful for measuring electrical resistivity at low temperatures. In Bi-Pb and Bi-Te alloys lead and tellurium will be about 0.02 and 0.2% respectively. Several spectrographic methods<sup>1-9</sup> have been reported for the analysis of bismuth. Of these, only in two methods<sup>3,5</sup> the determination of both Pb and Te has been reported. The method due to Yudelevich *et al.*<sup>3</sup> employs two different excitation conditions, the spark for determining Pb and the a.c. arc for Te. Konoalov *et al.*<sup>5</sup> reported the determination of Pb and Te at ultra trace levels in high purity bismuth. We have developed in our laboratory a spectrographic method to analyse Pb and Te in Bi-Pb and Bi-Te alloys respectively using a single excitation condition, viz., the d.c. arc.

TABLE I  
Experimental details

Spectrograph	Hilger large quartz, model E.492
Wavelength range	2300-3050 Å
Source to slit distance	38 cm
External optical system	Hilger 1025 lens in front of the slit
Filter	10% step filter
Electrode assembly	1/8" dia (U.C.C.) graphite electrode, pointed
Upper electrode (Cathode)	
Lower electrode (Anode)	1/4" dia (U.C.C.) graphite electrode with a cavity of depth 1.5 mm to contain 15 mg of sample or standard
Excitation source	D.C. arc 10 at amp
Analytical gap	4 mm
Slit width	15 microns
Exposure time	20 seconds
Photographic emulsion	Ilford N-30 ordinary
Emulsion calibration	Iron arc was used with the rotating seven step sector having a ratio of 2 : 1 between successive steps. Fe line at 2442.6 Å was used for calibration
Photographic processing	Developed in Kodak D-19 developer for 3 minutes at 20°C and fixed in Kodak F-5 fixer for 15 minutes
Densitometer	Hilger non-recording model L.451.9

About 1 g of the sample either Bi-Pb alloy or Bi-Te alloy was taken in a teflon vial and thoroughly ground in a mixer/mill\*. A portion of the sample was then mixed and ground with specpure conduct-

ing graphite powder (M/s. Ultra Carbon Corporation, USA) in the ratio 1 : 1 by weight. Fifteen mg of this mixture were packed in the cavity of a graphite electrode and excited in a d.c. arc. Complete experimental details are given in Table I. The spectra of the sample were compared with those of synthetic standards. Standards were prepared by grinding together appropriate amounts of specpure lead oxide and tellurium metal with bismuth metal (M/s. Johnson & Matthey & Co., UK) in an agate mortar. The concentration of Pb and Te in the standards ranged from 0.002-0.1% and 0.02-0.5% respectively.

The Pb line at 2614.2 Å and the Te line at 2385.8 Å were chosen as analytical lines with Bi lines at 2582.2 Å and 2448.1 Å respectively as internal standard lines. The working curves (log intensity ratio vs. log concentration) were plotted for the analytical line pairs Pb 2614.2 Å/Bi 2582.2 Å and Te 2385.8 Å/Bi 2448.1 Å and these were used to determine Pb and Te in the respective alloys. The precision of the method which is expressed as coefficient of variation was 11% for Pb determination and 10% for Te determination.

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\* Model No. 5100; M/s. Spex Industries, USA.

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### DIELECTRIC CONSTANTS AND EFFECTIVE IONIC CHARGES OF STRONTIUM SULPHIDE AND BARIUM SULPHIDE

IN an earlier communication<sup>1</sup> from this laboratory, the calculation of the effective ionic charges of the alkaline earth oxides was reported. It is felt that similar calculations for the alkaline earth sulphides would be of interest. However, meagre data are available on the dielectric properties of these sulphides. Due to unavailability of single crystals of the necessary size, measurements on single crystals could not be made. Recently, Drofenik and Azman<sup>2</sup> measured the static (low frequency) dielectric constants on powder samples of the alkaline earth sulphides. However, their results are approximate inasmuch as they were not corrected to theoretical density. Besides, Drofenik and Azman<sup>2</sup> have themselves pointed out the possibility of large errors in their value for barium sulphide due to carbon contamination. We have carried out measurements of the low frequency dielectric constants of powder samples of SrS and BaS. The results have been corrected for theoretical density by an accurate procedure and the final data have been employed to evaluate the effective ionic charge from Szigeti's<sup>3</sup> theory.

Die-pressed cylindrical samples 2.5 cm in diameter and 2-3 mm thick were prepared from pure chemicals supplied by Reidel de Hahn. Their packing fraction varied from 0.5 to 0.9. The dielectric constants were measured with the help of a Marconi Circuit Magnification Meter in conjunction with a special Marconi jig. The measurements were made at frequencies varying from 50 Kc/s to 50 Mc/s. The dielectric constant measured at these frequencies is generally taken as the static dielectric constant. At these frequencies, the dielectric constant should be frequency independent<sup>4</sup>. However, we observed a spurious frequency-dependence at the lower frequencies. Typical frequency vs. dielectric constant curves are shown in Fig. 1. Fortunately, this anomalous frequency-dependence ceased beyond 5 Mc/s and the frequency-independent dielectric constant at these frequencies is taken as the static dielectric constant. We may mention, in passing, that such anomalous frequency-dependence has been observed by Chaudhary and Rao<sup>5</sup> in the study of some oxides and by Axe *et al.*<sup>6</sup> in the case of cadmium fluoride; lattice imperfections are believed to be the cause of this effect.

Several procedures are available for obtaining the dielectric constant  $\epsilon$  of the solid from the measured dielectric constant  $\epsilon$  for a powder sample

of packing fraction  $\delta$ . We have used the following relation<sup>7</sup>:

$$\epsilon = (1/\delta^2) [(\epsilon_p - A)^{1/2} - (1 - \delta)(1 - A)^{1/2}]^2 + A$$

where A is a constant with a value 0.5.

The values of the static dielectric constants  $\epsilon$  of SrS and BaS are given in Table I along with those given by Drofenik and Azman<sup>2</sup>. There is some difference between our values and those given by Drofenik and Azman. In view of this difference, we have recalculated the effective ionic charge from the Szigeti equation<sup>3</sup>:

$$q^* = (W_0/Z_e)(3/n^2 + 2)(mv)^{1/2}(\epsilon - n^2/4\pi)^{1/2}$$

where the various quantities have the same significance as discussed in ref. (1). To facilitate comparison with the  $q^*$  values obtained by Drofenik and Azman, values for all other quantities are taken from ref. (2). The  $q^*$  values are given in Table 1.

TABLE I  
Dielectric constants and Szigeti charges of  
SrS and BaS

Crystal	Dielectric constant ( $\epsilon$ )		Szigeti charge ( $q^*$ )	
	Present work	Ref. 2	Present work	Ref. 2
SrS	8.5	7.6	0.58	0.52
BaS	9.1	11.0	0.49	0.60

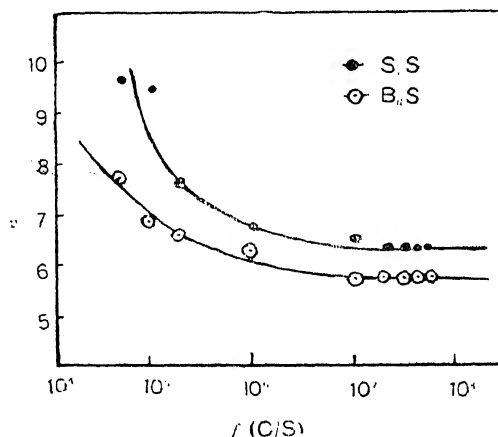


FIG. 1. Plot of frequency ( $f$ ) against the dielectric constant  $\epsilon_p$  of powder samples of SrS ( $\delta = 0.8$ ) and BaS ( $\delta = 0.7$ ).

Two features deserve to be noted. The value of  $q^*$  is of the order of 0.50 for both the salts. This is smaller than the values for the alkali halides<sup>4</sup>, indicating a lower ionicity. Also, there is a small but significant difference in our values of  $q^*$  for SrS and BaS, the value for BaS being lower than the value for SrS. This indicates that the ionicity of BaS is less than that of SrS, contrary to what

is expected from electronegativity considerations. It may be recalled that a similar effect has been observed in the alkaline earth oxides<sup>1</sup>.

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# NEAR ULTRAVIOLET ABSORPTION SPECTRA OF 2, 3, 5- AND 2, 4, 5-TRICHLOROPHENOL IN VAPOUR PHASE

THE effect of substitution of halogens on the electronic spectra of phenol has been studied by a number of workers<sup>1-2,5</sup>. With the aim to study the electronic spectra of 2, 3, 5- and 2, 4, 5-trichlorophenol (hereafter referred as 2, 3, 5-TCP and 2, 4, 5-TCP respectively) the present investigation has been undertaken. This is part of our programme designed to determine the ground and excited state fundamentals and to study the spectral shift produced in these molecules.

The spectra have been photographed on Hilger medium quartz spectrograph on Kodak N 30 plate. The length of absorbing column was 50, 100 and 150 cm and temperature was varied from 20° to 90°. About 30 bands in 2, 3, 5-TCP and 45 bands in 2, 4, 5-TCP have been observed. The bands are degraded towards red.

It has been established that  $A_{1g} - B_{2u}$  the vibronic transition of benzene which appears around 2600 Å shifts towards longer wavelength upon substitution. Further the vibrational frequencies are modified. The amount of shift and the change in the vibrational frequencies depends upon the nature and the position of the substituents.

In the molecules of the present study which are basically tetra-substituted benzene if we assume that OH group behaves as a single unit,  $C_s$  point group can be ascribed to them. Under, this reduced  $C_s$  symmetry the  $A_{1g} - B_{2u}$  vibronic transition of benzene becomes  $A' \rightarrow A'$  which is an allowed transition and the band systems obtained in the molecules under investigation are due to this transition ( $A' \rightarrow A'$ ). The most intense band at 2861·696 Å (34934  $cm^{-1}$ ) and 2949·172 Å (33897  $cm^{-1}$ ) has been taken as the O-O band in the respective molecules. Separation of some of the prominent bands agree fairly well with the infrared frequencies. These are taken as fundamental frequencies of ground and excited states. The various ground and excited state fundamentals determined on the basis of intensity and combinability and correlation with other benzene derivatives have been given in Table I. The whole of the spectra have been assigned with these frequencies forming combination with each other and with v-v transitions in each of the molecules. The (O-O) bands of phenol<sup>4</sup> and some TCP's are given in Table II. The red shift produced in these molecules are in order of 2, 4, 5-TCP > 2, 4, 6-TCP (3) > 2, 3, 5-TCP. The counterpart of the totally symmetric  $a_{1g}$  (992) vibration of benzene appears at 921  $cm^{-1}$  in the excited state. This vibration is substituent

TABLE I

2, 3, 5-TCP			2, 4, 5-TCP			Assignment
G.S.		E.S.	G.S.		E.S.	
I.R.	U.V.	U.V.	I.R.	U.V.	U.V.	
..	..	..	..	119	..	C-Cl O.P. bending
..	173	180	..	207	173	
415	..	362	410	..	241	Components of $e_{2g}$ (606)
580	..	496	570	..	406	
..	..	..	..	..	515	C-Cl stretching
840	..	800	850	..	644	Ring breathing
1130	..	1070	1145	..	1068	C-H planar bending
..	..	..	1295	..	1249	C-OH stretching



TABLE II

Molecules	Position of the (O, O) bands in $\text{cm}^{-1}$	Shift with respect to phenol in $\text{cm}^{-1}$
Phenol	36351	..
p-Chlorophenol	34820	1531
2, 3, 5-TCP	34934	1417
2, 4, 6-TCP	34026	2225
2, 4, 5-TCP	33897	2454

sensitive and its value reduces on substitution. This particular vibration appears strongly with reduced magnitude, i.e., less than  $921\text{ cm}^{-1}$  in the present study in excited state. The frequency of C-OH stretching vibration does not change much on excitation. Similar observation has been made by previous workers on substituted<sup>4,5</sup> phenols.

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#### SPECTROPHOTOMETRIC AND ANALYTICAL STUDIES OF Fe (III) COMPLEXES WITH 3-PHENYL-4, 5, 7-TRIHYDROXYCOUMARIN AND 4, 6-DIHYDROXY-3', 4'-DIMETHOXYAURONE

Fe (III) complexes with 3-phenyl-4, 5, 7-trihydroxycoumarin and 4, 6-dihydroxy-3', 4'-dimethoxyaurone have been studied spectrophotometrically by three methods, namely, monovariation, continuous variation and slope-ratio methods. The complexes are found to possess molar ratios (metal: ligand) of 1:1 and 1:2 respectively. The nature and the composition of the complexes have further been established by analytical and spectral studies.

3-phenyl-4, 5, 7-trihydroxycoumarin was found earlier by us as a good complexing reagent for various metal cations<sup>1</sup> and its complex with U (VI) was investigated spectrophotometrically<sup>2</sup>. The complex was found to have a stoichiometry of 1:1 (metal: ligand). Following this investigation, Bharat *et al.*<sup>3</sup> have also reported complex formation of a related compound 3-phenylazo-4-hydroxycoumarin with various metal ions. Later 4, 6-dihydroxy-3', 4'-dimethoxyaurone was equally good for complexing some metal ions<sup>4</sup>. Its complex with U (VI) was investigated spectrophotometrically and analytically and found to have a stoichiometry of 1:2 (metal: ligand). Since the two organic compounds give intense coloration with Fe (III) cation

it was decided to investigate the complex formation in each case.

**Experimental.**—Analar ferric chloride was used for the preparation of standard solution of iron. It was estimated by the usual analytical procedure<sup>6</sup>. 3-Phenyl-4, 5, 7-trihydroxycoumarin was prepared by the method of Gilbert *et al.*<sup>7</sup> and purified by repeated crystallization from ethanol until it gave a single spot on T.L.C. and a constant melting point<sup>2</sup>. 4, 6-dihydroxy-3', 4'-dimethoxyaurone was prepared according to the method given by Jain *et al.*<sup>8</sup> and King *et al.*<sup>9</sup> and purified by repeated crystallization until it gave a single spot on T.L.C. [solvent system of ethyl formate, formic acid and toluene (20:6:25)] and a constant melting point of  $220^\circ$  (Lit.<sup>8</sup>  $220-21^\circ$ ). Standard solutions of both the ligands were prepared in 95% ethanol. Optical density measurements were carried out with Beckman DB Spectrophotometer and the pH measurements were made with Philips PR 9405 L pH meter. Ligand solutions of appropriate strength were employed as reference in order to correct the observed optical density for no reaction of the constituents. The corrected values were plotted.

Job's method of monovariation<sup>10</sup> and continuous variations as modified by Vosburgh and Cooper<sup>11</sup> and the slope-ratio method of Harvey and Mannings<sup>12</sup> were employed for ascertaining the molar ratios of the complexes. In the spectrophotometric investigation of Fe(III)-3-phenyl-4, 5, 7-trihydroxycoumarin complex  $1 \times 10^{-3}$  M solutions of the metal ion and the ligand was used in each of the methods and the optical density measurements were carried out at 590 nm at a pH of  $3.8 \pm 0.2$ . Similarly, in the spectrophotometric investigation of Fe(III)-4, 6-dihydroxy-3', 4'-dimethoxyaurone complex,  $0.5 \times 10^{-3}$  M solutions of Fe(III) and the aurone derivative were used in the monovariation and continuous variation methods and  $0.25 \times 10^{-3}$  M solutions in the slope-ratio method. The optical density measurements were carried out at 490 nm. As the complex was stable in the pH range 1.8 to 4.4, the pH was maintained at 4.0.

The metal complexes were prepared in the solid state by mixing the methanolic solutions of ferric chloride and the respective ligands in the molar ratios determined spectrophotometrically and the solutions on concentration yielded the respective complexes. They were purified by repeated crystallization and dried over anhydrous calcium chloride in vacuum. Iron was estimated as  $\text{Fe}_2\text{O}_3$  by ignition.

#### DISCUSSION OF THE RESULTS

##### *Fe(III) Complex with 3-Phenyl-4, 5, 7-Trihydroxycoumarin*

The observations show that the molar ratio of the complex of Fe(III) ion with the coumarin

derivative is 1 : 1. This bluish green complex is stable in the pH range 3.8 to 6.00 ( $\pm 0.2$ ). Above pH 6.0, the complex shows a colour change. It turns wine-red in colour. The composition of the wine-red complex and the possible use of the blue-green complex as an acid-base indicator are under study. In the infra-red spectrum, 3-phenyl-4, 5, 7-trihydroxycoumarin shows the carbonyl frequency at  $1650\text{ cm}^{-1}$  and a hydroxyl band at  $3520\text{ cm}^{-1}$  which shift appreciably in the complex to  $1600\text{ cm}^{-1}$  and  $3430\text{ cm}^{-1}$  respectively. It indicates that out of the two possible tautomeric forms of the coumarin derivative, the isoflavone form is stabilized during complexation.

The formation of the solid complex may be represented as follows:

$\text{Fe}^{3+} + \text{C}_{15}\text{H}_{10}\text{O}_5 + 4\text{CH}_3\text{OH} \rightarrow [\text{Fe}(\text{C}_{15}\text{H}_9\text{O}_5)(\text{CH}_3\text{OH})_4]^{2+} + \text{H}^+ + [\text{Fe}(\text{C}_{15}\text{H}_9\text{O}_5)(\text{CH}_3\text{OH})_4]\text{Cl}_2$  requires Fe, 10.5%; C, 43.5%; H, 4.8%. Found: Fe, 10.5%; C, 44.7%; H, 5.5%. It is possible that the metal ion is coordinated with the oxygens of the 4-carboxyl and 5-hydroxy groups of the isoflavone form.

#### Fe (III) Complex with 4.8-Dihydroxy-3' 4'-Dimethoxy-aurone

The observations show that the molar ratio in this complex is 1 : 2 (metal : ligand). The solid complex is chocolate-brown in colour and is stable in the pH range 1.8 to 4.4. Its formation may be represented as:

$\text{Fe}^{3+} + 2\text{C}_{17}\text{H}_{14}\text{O}_6 + 2\text{CH}_3\text{OH} \rightarrow [\text{Fe}(\text{C}_{17}\text{H}_{13}\text{O}_6)_2(\text{CH}_3\text{OH})_2]^{+} + 2\text{H}^+$   
[Fe (C<sub>17</sub>H<sub>13</sub>O<sub>6</sub>)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] Cl<sub>2</sub> requires C, 55.2%; H, 4.3%; Fe, 7.1%; Found: C, 54.2%; H, 4.4%; Fe, 6.8%. Analogous to the coumarin complex, the metal ion can be expected to coordinate with the oxygen atoms of the 3-carbonyl and 4-hydroxy groups of the ligand.

To establish the structure of the complexes, further investigations are in progress.

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### STUDIES IN S<sup>IV</sup>-S<sup>VI</sup> SYSTEM

KINETICS of oxidation of Dimethyl sulphoxide by Mn<sup>+</sup> has been reported earlier wherein it has been observed that the order with respect to substrate and oxidant is unity<sup>1</sup>.

This communication deals with kinetics of oxidation of DMSO by Cr<sup>VI</sup> in aqueous medium. The reaction is first order in oxidant, and second order in acidity. Till a substrate concentration of 1.55 M the order with respect to DMSO is one. But above this concentration, dependence on DMSO changes over to two: The kinetics were followed by the usual iodometric method. Rate constants reported are computed from the disappearance of Cr (VI).

TABLE I

Cr<sup>VI</sup> = 0.003 M, DMSO = 2.82 M, Temp. 35° C.

Acidity, M	H <sub>0</sub>	$k_1 \times 10^4 \text{ min}^{-1} \text{ HClO}_4$	$\text{H}_2\text{SO}_4$
0.125	2.19	3.358	9.21
0.188	1.99	10.96	20.32
0.25	1.79	23.62	41.87
0.313	1.62	46.06	72.93
0.5	1.46	211.0	216.0

H<sub>0</sub> values used for dependence on acidity have been determined by Photoelectric Colorimetry using P-NO<sub>2</sub> aniline as indicator (PkbH<sup>+</sup> = 0.99).

TABLE II

Cr<sup>VI</sup> = 0.003 M, HClO<sub>4</sub> = 0.5 M, Temp. 35° C.

DMSO, M	$k_1 \times 10^4 \text{ min}^{-1}$
2.82	211.0
2.115	124.4
0.7052	20.00
0.3526	10.00
0.1410	4.00

Effects of Mn<sup>++</sup> (concentration = 0.00253 M,  $k_1 = 0.0009541 \text{ min}^{-1}$ ) and pyridinium persulphate (Conc. = 0.00422 M,  $k_1 = 0.0008529 \text{ min}^{-1}$ ) are also studied at 0.5 M HClO<sub>4</sub> and DMSO = 0.3526 M, and found to be marginal.

But the effects of dipyridyl and orthophenanthroline are marked and the reactions are well accelerated. The reaction mixture develops a light pink colour indicating a  $[\text{Cr}(\text{dipy.})_3]^{+2}$  complex which dismutates later to a stabler system<sup>2</sup>.

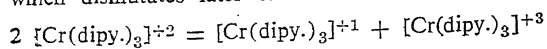


TABLE III

$\text{Cr}^{VI}=0.003 \text{ M}$ ,  $\text{HClO}_4=0.5 \text{ M}$ ,  $\text{DMSO}=0.3526 \text{ M}$ ,  
Temp.  $35^\circ \text{C}$ .

Dipyridyl, M	$k_1 \times 10^4 \text{ min}^{-1}$
0.00376	548.4
0.0016	219.8
0.00079	74.17

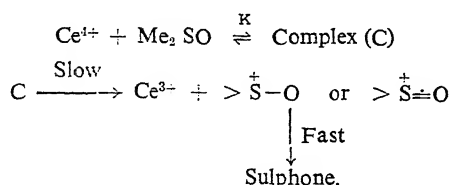
Orthophenanthroline	$k_1 \times 10^4 \text{ min}^{-1}$
0.003817	368.4
0.00161	115.2
0.00091	20.15

The reactions are uniformly faster in  $\text{H}_2\text{SO}_4$  medium, acidity dependence in  $\text{H}_2\text{SO}_4$  also being two.

The mechanism of oxidation is presumably by electron transfer from the sulphoxide to  $\text{Cr}^{VI}$  followed by the attack of the sulphur radical on the  $\text{Cr}=\text{O}$  bond of the oxidant leading to the sulphone. This is quite possible as similar oxygen transfer has been postulated by isotopic labelling in the sulphide oxidation to sulphoxide<sup>3</sup>.

Kinetics of oxidation of DMSO by  $\text{Ce}^{+4}$  has also been carried out. The reaction is first order in oxidant and first order in substrate. Below  $0.5 \text{ M}$  substrate concentration, the reaction rate is unaffected by concentration variation. Above  $0.5 \text{ M}$  DMSO retardation has been observed, which is due to the formation of unreactive stable complexes at higher concentrations of DMSO. Such retardation in  $\text{Ce}^{+4}$  oxidations with increase in substrate concentration has been reported earlier<sup>4,5</sup>.

It appears that  $\text{Ce}^{IV}$  oxidation of DMSO might go through complex formation followed by rate determining electron transfer forming the radical ion.



Such radical ions have been postulated in the  $\text{Mn}^{3+}$  oxidation of DMSO (*loc. cit.*).

TABLE IV

$\text{Ce}^{+4}=0.02 \text{ M}$ ,  $\text{HClO}_4=0.5 \text{ M}$ , Temp.  $45^\circ \text{C}$ .

DMSO, M	$k_2 \times 10^3 \text{ l. mole}^{-1} \text{ min}^{-1}$
2.82	6.187
2.115	10.1
1.41	21.2
1.2692	25.9
1.2578	28.96
0.7052	40.51
0.4936	48.14
0.3526	52.29

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#### LIPIDS OF MANGO (*MANGIFERA INDICA*)

It has been reported by Gholap and Bandopadhyaya<sup>1</sup>, that lipids from the pulp of mango consist, predominantly of triglycerides, the fatty acid composition of which differs with varieties, and that the aroma and the flavour of the pulp are influenced by palmitic and palmitoleic acid distribution in the lipids. The fatty acid composition of the pulp of a popular variety of mango, Alphonso, has also been reported by Gholap, Bandopadhyaya and Sreenivasan<sup>2</sup>. The present communication contains a preliminary study of lipids of the pulp and peel fractions of five varieties of mangoes popular in Andhra Pradesh.

Table ripe mangoes were washed with distilled water. The peels and then the pulps were separated from the stone, with a stainless steel knife. These were then homogenised separately, in a blender.

TABLE I

Lipid content of peel and pulp of mango

Variety	% of lipid in peel	% of lipid in pulp
Malgoa	1.17	0.80
Totapuri	0.75	1.12
Benishan	0.88	0.80
Nelam	1.70	..
Sundari	0.98	1.36

These were extracted separately with 100 volumes of *i*-propanol and the residues again extracted with 100 volumes of Chloroform: *i*-propanol (1:1).

according to the procedure of Nicholas<sup>3</sup>. The extracts were combined separately, evaporated under reduced pressure (55° C), and the residues thus obtained were subjected to Folch procedure<sup>4</sup>, for effective elimination of the non-lipids. The Chloform layer thus obtained, was evaporated under vacuum (55° C) and finally in a vacuum desiccator over sulphuric acid.

A silica gel plate (20 × 20 cm, 250 μ) was cleaned by a pre-run with Chloform, activated at 110° C, and the samples (10 μg/μl) were spotted along with authentic samples of lipids. The chromatogram was developed by the two step thin layer chromatographic procedure of Biezenski<sup>5</sup>, air-dried and visualised by exposure to iodine vapours.

The yields of lipids from the pulps and peels of the five varieties of mango are tabulated in the accompanying Table I. The values are considerably higher than reported by Nandi<sup>6</sup> (0.073–0.156%) and other workers<sup>7</sup> (0.03–0.92%). These differences might have arisen due to varietal and ecological factors, as well as the procedures adopted in the extractions of the lipids. It is noteworthy that the peels of the fruits of Malgoa and Benishan varieties are richer in lipids than their pulps, which is reverse in the case of Totapari and Sundari varieties.

It is evident from the chromatogram (Fig. 1) that the predominant components of the liquids of

mango pulp and peel are triglycerides and/or esters and/or hydrocarbons (solvent front and close to the solvent front). In addition, the peel and pulp of the varieties of mangoes reported here seem to contain a variety of lipids, the intricate nature of which calls for further investigation.

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#### POTENTIOMETRIC STUDY OF METAL COMPLEXES OF N-o-ETHOXY BENZOYL-PHENYLHYDROXYLAMINE

ALTHOUGH a large number of analytical works have been reported<sup>1</sup> for chelating properties of various N-substituted phenylhydroxylamines, studies on the physico-chemical properties of their metal complexes are limited<sup>2-4</sup>. It was therefore thought worthwhile to undertake a systematic study of the effect of substitution in some of these reagents on the stability of their metal chelates. The present communication constitutes a potentiometric investigation of Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) chelates of N-o-ethoxy benzoyl-phenylhydroxylamine in 50% v/v aqueous dioxane medium at μ = 0.1 M (NaClO<sub>4</sub>) and t = 25 ± 0.1° C to ascertain the chelating behaviour of this reagent and to correlate the stability data with various properties of the metal. The order of stability is found to be Cu > Ni > Co > Zn > Mn.

*Experimental.*—Dioxane (AnalaR, B.D.H.) was further purified by the method described by Weissberger<sup>5</sup>. Solutions of carbonate free sodium hydroxide and perchloric acid (E. Merk) were standardized in the usual way. Perchlorates of

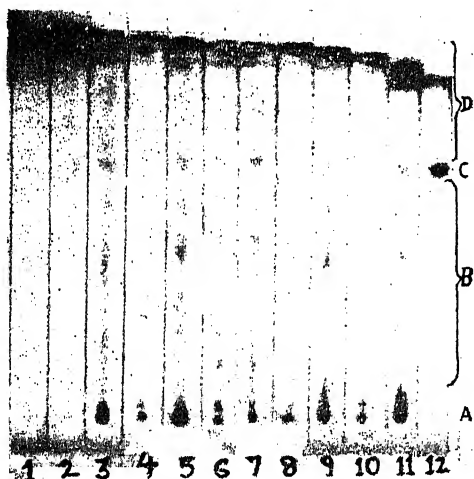


FIG. 1. Separation of lipids of peel and pulp of varieties of mango by two step thin layer chromatography. 1, Methyl palmitate; 2, Hydrogenated oil; 3, Malgoa peel; 4, Malgoa pulp; 5, Totapari peel; 6, Totapari pulp; 7, Benishan peel; 8, Benishan pulp; 9, Sundari peel; 10, Sundari pulp; 11, Neelam peel; 12, Cholesterol.

A, Origin and phospholipids; B, Monoglycerides; C, Cholesterol; D, 1, 2-diglycerides, 1, 3-diglycerides, free fatty acids, triglycerides, cholesteryl esters and hydrocarbons (in ascending order)<sup>5</sup>.

Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) (Fluka) were dissolved in known volume of perchloric acid and the metal contents were determined in the conventional way. Sodium perchlorate solution was prepared from neutral  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$  (Riedel). For the preparation of *N*-*o*-ethoxy benzoyl-phenylhydroxylamine, earlier method was followed<sup>1</sup>. Redistilled water was used in all cases.

All pH measurements were made with a Cambridge bench type pH-meter equipped with a glass-saturated calomel electrode pair and standardized with buffer solutions at pH 4.0 and 9.0. The design of the glass jacketed titrating vessel was such that it allowed nitrogen (presaturated with solvent) to be passed through the reaction mixture and enabled measurements to be carried in an atmosphere of nitrogen. A magnetic stirrer was employed for stirring the solutions whose temperature was kept constant by an electrically maintained thermostat.

**Potentiometric titration.**—Calvin-Bjerrum<sup>6,7</sup> pH titration technique was used for titrating the ligand with standard sodium hydroxide solution (0.1 M) in the absence of, and in the presence of, the metal ions to be studied. These included titrations of (i)  $\text{HClO}_4$  ( $5.0 \times 10^{-4}$  moles) + ligand ( $2.0 \times 10^{-4}$  moles) and (ii)  $\text{HClO}_4$  ( $5.0 \times 10^{-4}$  moles) + ligand ( $2.0 \times 10^{-4}$  moles) + metal ion ( $2.0 \times 10^{-5}$  moles). The ionic strength was maintained constant at 0.1 M by addition of sodium perchlorate solution. The initial volume of the reaction mixture was 100 ml aqueous dioxane containing 50% dioxane by volume. The pH-meter readings obtained after each equilibrium point at a given alkali addition in duplicate titrations were reproducible with a maximum variation of 0.02 pH unit. Necessary pH correction<sup>8</sup> was made for using 50% v/v dioxane-water mixture.

**Calculations.**—The reagent studied in the present work is a weak acid of the type HR. Acid dissociation constant,  $K_{\text{OH}}$ , was calculated from the experimental points in the buffer region using the equation:

$$K_{\text{OH}} = \frac{H^+ \cdot S_1}{T_R - S_1}$$

where,

$$S_1 = H^+ + Na^+ - OH^- - A.$$

$T_R$  and A were the total reagent and perchloric acid concentrations, respectively. Concentration sign, square brackets are omitted for clarity. Calvin-Bjerrum method was utilised for calculating chelate formation constants from the following equations:

$$\bar{n} = \frac{1}{T_M} \left( T_R - S_2 \cdot \frac{K_{\text{OH}} + H^+}{H^+} \right)$$

$$R^- = \frac{K_{\text{OH}} \cdot S_2}{H^+}$$

where,

$$S_2 = T_R + A + OH^- - Na^+ - H^+$$

$T_M$ ,  $\bar{n}$  and  $R^-$  represented total metal ion concentration, average number of chelating agent bound to one metal ion and anionic chelating agent concentration, respectively. The stepwise chelate formation constants,  $\log K_1$  and  $\log K_2$ , were obtained from the plot of  $\bar{n}$  against  $PR^-$  ( $PR^- = -\log R^-$ ) at values  $\bar{n} = 0.5$  and 1.5, respectively.

**Results and Discussion.**—The value of  $\bar{n}$  obtained in Mn (II), Co (II), Ni (II), Cu (II) and Zn (II) systems was above 1.5 before the onset of precipitation with *N*-*o*-ethoxy benzoyl-phenylhydroxylamine. The possibility of hydrolysis of these metal ions in presence of excess of the ligand is ruled out, as there was no possible indication in the trend of pH changes during titrations. Moreover, the hydrolysis curves of these metal ions studied<sup>9</sup> in the same solvent medium show that the stability measurement presented here is not likely to interfere with the metal ion hydrolysis. The oxidation of Co (II) to give complex of the type  $\text{CoR}_3$  by the release of three protons seems unlikely in view of the similar nature of the formation curve of this metal chelate with the rest. Further the results (Table I) do not indicate any reversal of stability order in the case of Co (II) complex. Therefore, from the above arguments it may be concluded that only pure complexes of the type  $\text{MR}^+$  and  $\text{MR}_2$  were formed in solution. The values of  $\log K_1$  and  $\log K_2$  for Co (II), Ni (II), Cu (II) and Zn (II) chelates were directly read from the formation curves (Fig. 1). However, in

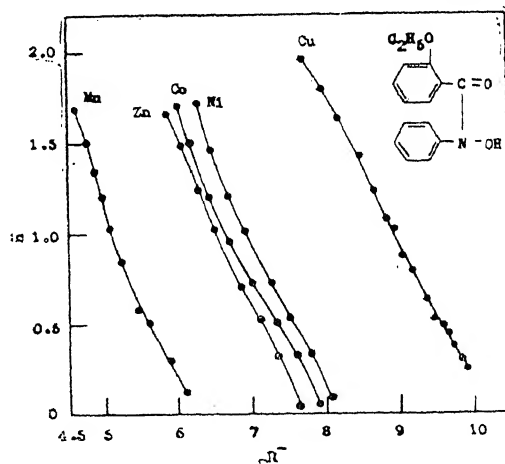


FIG. 1. Metal-ligand formation curves.

case of Mn (II) chelate, where  $\log K_1$  and  $\log K_2$  differed by less than one unit, the method of least squares<sup>10</sup> was employed to calculate these values. Results are shown in Table I.

TABLE I

Stability constants of *N*-*o*-ethoxy benzoyl-phenylhydroxylamine metal chelates $\mu = 0.1 \text{ M (NaClO}_4\text{)}$   $t = 25 \pm 0.1^\circ \text{C.}$ 

Metal	log $K_1$	log $K_2$	log $\beta_2$
Cu	9.55	8.32	17.87
Ni	7.45	6.40	13.85
Co	7.32	6.17	13.49
Zn	7.15	6.05	13.20
Mn	5.50	4.82	10.32

The experimental pK value of *N*-*o*-ethoxy benzoyl-phenylhydroxylamine is 10.35. A comparison of the pK value of *N*-*o*-ethoxy benzoyl-phenylhydroxylamine with the parent compound<sup>11</sup>, *N*-benzoyl-phenylhydroxylamine reveals that ethoxy group in ortho position of the benzene ring have increased the pK value by 0.3 pK unit. In the former case, +M effect of the ethoxy group is responsible for an increased pK value. With this increase in the pK value of ethoxy compound, the stabilities of its metal chelates also correspondingly increase. The order of stability with respect to log  $K_1$  or log  $\beta_2$  is found to be Cu > Ni > Co > Zn > Mn which is in general agreement with the stability order reported<sup>12,13</sup>. The validity of the linear relationship between log  $K_n$  of the metal chelates and second ionisation of the metal ions was examined. An approximate straight line relationship holds good (Fig. omitted). However, the correlation between log  $K_n$  and atomic number is all the same that was observed previously<sup>13</sup>.

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#### FORMATION OF AZINES FROM 4-ALKYL-4-TRIALO-ALKYL-2, 5-CYCLOHEXADIENONES

In an earlier publication<sup>1</sup> we have reported some of the properties and reactions of 4-alkyl-4-trialoalkyl, cyclohexadienones. We now describe here the formation of azines by the reaction of the latter with hydrazine hydrate in boiling alcohol (3 hr) containing a drop of concentrated hydrochloric acid.

	R	R <sub>1</sub>	R <sub>2</sub>	m.p.
I	CH <sub>3</sub>	CHCl <sub>2</sub>	H	166–68°
II	Et	CCl <sub>3</sub>	H	190–91°
III	C <sub>6</sub> H <sub>5</sub>	CCl <sub>3</sub>	H	162–63°
IV	CH <sub>3</sub>	CCl <sub>3</sub>	CH <sub>3</sub>	144–46°
V	CH <sub>3</sub>	CBr <sub>3</sub>	H	207–209°
VI	CH <sub>3</sub>	CBr <sub>3</sub>	CH <sub>3</sub>	157–58°

In the formation of VI, methanol was used as ethyl alcohol was found to give a dark tarry material. The azines were found to be quite stable and on reduction with sodium borohydride or lithium aluminium hydrate were recovered unchanged. Catalytic reduction of the azines with Raney nickel or palladium charcoal produced dark compounds.

The azines were usually orange compounds crystallized from a mixture of chloroform-alcohol or petroleum ether-chloroform. The u.v. spectrum showed  $\lambda_{\text{max}}^{\text{MeOH}}$  240 (log  $\epsilon$  3.908) and 340 nm (log  $\epsilon$  4.599) whereas the i.r. spectrum (Nujol) showed bands at 1660 cm<sup>-1</sup> corresponding to C=N.

When the azine I prepared from 4-methyl-4-dichloromethyl-2,5-cyclohexadienone by the action of *p*-cresol with chloroform and alkali as described in literature<sup>2</sup> was boiled in diglyme at 156° for 4 hours, 4,4'-dimethyl azobenzene (m.p. 144°) was isolated. Similarly, 3,3',4,4'-tetramethyl azobenzene<sup>3</sup> (m.p. 140) was obtained from IV.

The same reaction when repeated with the bromo compound V failed to give a pure substance. Institute of Science, J. R. MERCHANT.  
Madame Cama Road, DIPAK ROY.  
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### DETECTION OF SULPHIDE USING CACOTHELINE AS REAGENT

AMONG the various reagents which give sensitive tests for sulphide mention may be made about the use of  $\text{NaN}_3$  and  $\text{I}_2$  mixture<sup>1</sup> and alkali monochloro acetate<sup>2</sup>. The present communication deals with a simple and easy method with a cheaper reagent for the detection of sulphide.

**Cacotheline**<sup>3</sup>.—The cacotheline used in the investigation is the one of 99–99.5% purity. It is dried at 110°C for 1 hour. 0.2% solution of cacotheline is prepared and is further diluted to 0.02% to test at lower concentrations.

**Sodium sulphide**.—0.1 N solution of sodium sulphide (Pfizer) is prepared and further diluted to 0.01 N. It is standardised<sup>4</sup> by iodimetric method. All the reagents used were of analytical reagent grade quality.

**On the Spot Plate**.—0.05 ml of 0.2% cacotheline is placed in the cavity of a spot plate and 0.10 ml of 4.58 pH buffer solution is added and stirred well with a glass rod followed by 0.10 ml of test solution. A pink colour is developed which is stable upto 5 minutes.

**Limit of identification** : 0.39  $\mu\text{g}$  in a total volume of 0.25 ml.

**Limit of dilution** :  $1 : 6.4 \times 10^3$ .

The sensitivity of the test can be increased to the following limits by using solutions prepared with boiled distilled water and the addition of 0.05 ml of 1 M potassium antimonate (Analar B.D.H.).

**Limit of identification** : 0.22  $\mu\text{g}$  in a total volume of 0.30 ml.

**Limit of dilution** :  $1 : 3 \times 10^6$ .

Barium chloride, sodium chloride and potassium antimonate do not interfere in this procedure but  $\text{Pb}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{As}^{+++}$ ,  $\text{Hg}^{++}$ ,  $\text{Ni}^{++}$  and  $\text{Mn}^{++}$  will interfere.

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### CYTOLOGICAL EFFECTS OF THE ROOT EXTRACTS OF *TEPHROSIA HAMILTONII*

THE use of plant decoctions as fish poisons and insecticides dates back to the seventeenth century. The insecticidal properties of the root extracts of *Tephrosia* of Leguminosae having compounds related to rotenone have been described<sup>1,2</sup>. The aqueous root extract of *Tephrosia purpurea* (identified as *T. hamiltonii*<sup>3,4</sup>) has long been known as a medicine for tympanitis, dyspepsia and chronic diarrhoea<sup>5</sup>. The necessity of investigating the mutagenicity of biologicals has been recently pointed out<sup>6</sup> and this paper describes the effects of the root extract on the mitotic cells of *Allium cepa*.

The extract was prepared by crushing and leaving for 4 hours 25 gm of thoroughly washed roots in 800 ml distilled water and filtering it. While in one series roots growing vigorously from the bulbs of *A. cepa* were treated with the filtrate at room temperature for 4, 8, 12, 24 and 48 hours, in another series the 24 hour treated roots were allowed to recover in distilled water for 24 hours. The cytological preparations were made by haematoxylin squash technique<sup>7</sup>.

The predominant effect induced by the extract was one of C-mitotic action as evidenced by the appearance of the metaphase chromosomes with the contraction increasing with the period of treatment (Figs. 1–4). The other mitotic abnormalities were in the form of anaphase inhibition, chromosomal groupings (Fig. 5), induction of binucleate cells (Fig. 6), lagging of fragments (Fig. 7), stickiness (Fig. 8) and chromosome erosions in some instances. The partial spindle inhibition seen after 4 hour treatment was completed by 8–24 hours. An antimitotic action was obvious from the occurrence of very few divisional figures after 48 hour treatment. The recovery of treated roots showing the return to the normal condition demonstrated that the effects caused initially were reversible.

The C-mitotic action of a variety of agents has been discussed from time to time<sup>8,9</sup>. The sister chromatids of C-pairs observed here did not diverge so much apart as in the case of X-shaped configurations generally seen after treatment with colchicine or other substances. The investigations on the effects of rotenone on the Chinese hamster cells have revealed an increase in the mitotic index, metaphase arrest, dispersal of condensed chromosomes and disruption of the mitotic spindle<sup>10</sup>. This communication demonstrates that the crude root extracts of the plant containing substance(s) related to rotenone have produced almost the same effects

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indicating the possible hazards of such extracts used in medicine. The results are also suggestive of an utility of the extract as a simple means of treating the material for 4 to 8 hours to enable a chromosome analysis.

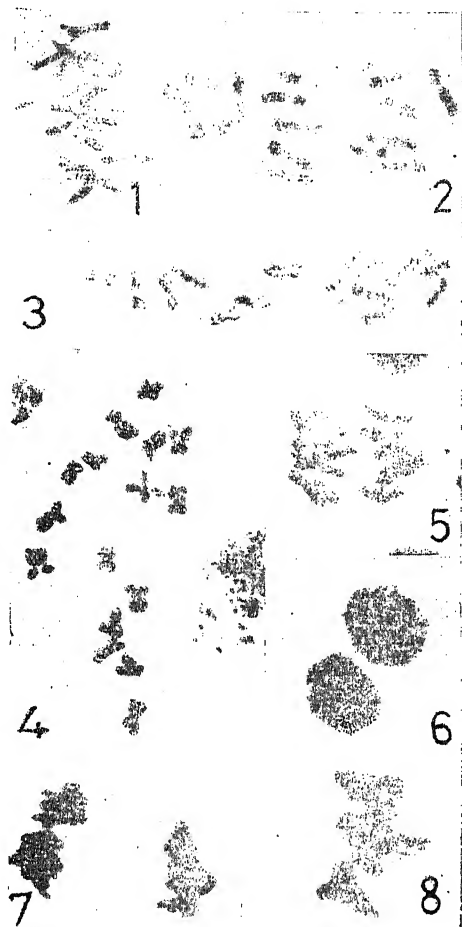
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FIGS. 1-8. Haematoxylin squashes of *A. cepa* roots. Fig. 1. Control. Figs. 2-8. Treatment with root extract of *T. hamiltonii*,  $\times$  ca. 950. Figs. 2, 5, 7 & 8. 8 hrs. Fig. 3. 12 hrs. Figs. 4 & 6. 24 hrs. Fig. 2. Straightened C-metaphase chromosomes showing constrictions. Fig. 3. Note the distinct centromere and chromatids in each chromosome. Fig. 4. C-metaphase showing an accentuation of chromosome contraction. Fig. 5. Two chromosomal groupings are lying side by side at metaphase. Fig. 6. A binucleate cell. Fig. 7. Lagging chromosome fragments. Fig. 8. A sticky metaphase.

The authors are thankful to Prof. O. S. Reddi, Department of Genetics, Osmania University, Hyderabad, for his encouragement.

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#### A NUCLEAR POLYHEDROSIS OF *DASYCHIRA MENDOSA* HB. (LEPIDOPTERA: LYMANTRIIDAE)

POLYHEDROSIS has been recorded in *Dasychira pudibunda* L. by Krausse (1919) and Urban (1967). Hukuhara *et al.* (1966) described a nuclear polyhedrosis virus affecting *Dasychira lacuples confusa* (Bremer). An epizootic of nuclear polyhedrosis of *Dasychira mendosa* Hb. was observed in Coimbatore during 1973. Large number of dead and dying caterpillars could be observed on all the castor plants present in an area of about one hectare. Several live caterpillars collected from the area also died of polyhedrosis in a couple of days. This appears to be the first record of nuclear polyhedrosis on *D. mendosa*.

Infected caterpillars were dirty brown in colour and soon after death, the skin became extremely fragile. Examination of tissue smears under the phase contrast microscope revealed polyhedra in the nuclei of fat body, hypodermis and tracheal matrix.

Diameter of 100 polyhedra ranged from  $0.792 \mu$  to  $2.816 \mu$  with an average of  $1.661 \mu \pm 0.011$ . Electron micrograph of sections of polyhedra showed



virions to be occluded in bundles of 2 to 13 (Fig. 1).

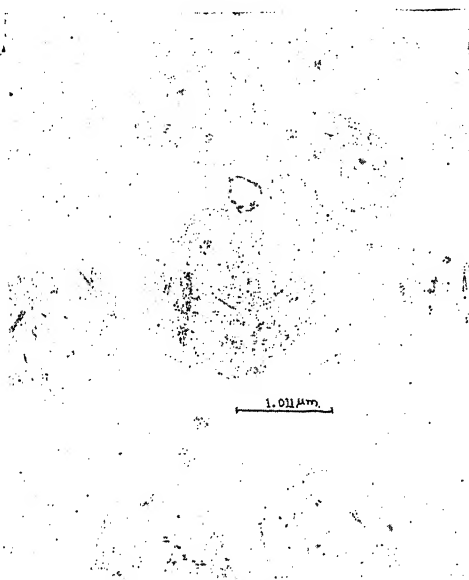


FIG. 1. Electron micrograph of sections of polyhedra of *Dasychira mendosa* showing virions occluded in bundles.

The authors are grateful to Dr. Jean R. Adams, Insect Pathology Laboratory, Beltsville, Maryland, for making the electron micrograph of the virus.

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## TWO NEW FAMILIES OF CHYTRIDS

THIS paper is concerned with the taxonomy of *Micromycopsis* Scherffel<sup>1</sup>, *Endodesmadium* Canter<sup>2</sup> and *Caulochytrium* Voos and Olive<sup>3,4</sup>, three Chytrid genera which seem unique in certain features.

*Micromycopsis* is currently classified in the Synchytriaceae<sup>5,6</sup>. Scherffel established the genus for two species, *M. cristata* Scherffel and *M. fischerii* Scherffel, both from Hungary, and on algae, the former on *Hyalotheca dubia*, and the latter on *Zygonium*. In both species, the thallus is said to function as a prosorus at maturity; the prosorus develops an exit tube of variable length through which the prosoral contents flow out to form an apical, epibiotic, or sometimes endobiotic, structure

that is equated with a sporangial sorus. The production of a prosoral tube was considered by Scherffel a feature distinctive enough for separation from the genus *Micromyces* Dangeard in which the sorus is sessile on the prosorus. This distinction is considered not tenable as in one species of *Micromyces*; *M. ovalis* Rieth, occasional development of a prosoral exit tube has been observed. Nevertheless, the two *Micromycopsis* species described by Scherffel are unique for other reasons. In *M. cristata*<sup>1,2</sup> the sporangia usually show amoeboid movement on liberation from the "sorus" and some of them have also been seen to have a flagellum. These amoeboid swimmers (sporangia) encyst and then produce posteriorly uniflagellate zoospores endogenously. In *M. fischerii*<sup>1,2</sup>, each sporangium in a sorus produces about five posteriorly uniflagellate zoospores that exhibit jerky and amoeboid movement before they encyst; each cyst then releases, from within, 2–6 smaller posteriorly uniflagellate swimmers that presumably germinate and infect the algal host again. Thus, in *M. fischerii* both "primary" and "secondary" zoospores are formed. Though both species are generally held to have the common feature of having sporangial sori and have even been merged with *Micromyces*<sup>6</sup>, what is interesting about these species is the fact that the "primary" swimmers encyst and then give rise to "secondary" swimmers. The "sorus" in *M. cristata* is here interpreted as a sporangium rather than a sorus and the so-called "sporangia" of this species are, in fact, zoospores, all of which may be posteriorly uniflagellate and yet, in the observations reported by Scherffel, could well have been in the process of encystment. If this is true, "primary" and "secondary" zoospore phases become the common characteristic of both species—a feature of sufficient import to lend validity to *Micromycopsis* as a genus distinct from *Micromyces*. A new family Micromycopsidaceae is therefore proposed to accommodate *Micromycopsis*.

*Endodesmadium formosum* Canter<sup>2</sup> (type species of the genus *Endodesmadium* Canter<sup>2</sup>) has close similarity to *Micromycopsis cristata*, a point stressed by Canter herself in her paper which, in fact, is a major contribution to our knowledge of these fungi. *E. formosum* is parasitic on certain algae: *Netrium oblongum*, and *Cylindrocystis crassa* and *C. brebissonii*. Canter placed *Endodesmadium* in the Synchytriaceae, the endobiotic, holocarpic thallus being interpreted in its development as a prosorus. However, the so-called "sporangia" from the sorus are sometimes posteriorly uniflagellate and it is, therefore, logical to assume that these sporangia are swimmers and the so-called sorus a sporangium—a situation clearly

reminiscent of that in *Micromyopsis cristata*, as already explained. A natural corollary to this conclusion would be that in *Endodesmidium formosum* also there are "primary" and "secondary" zoospore phases. If, now, the presence of a prosoral tube is considered a character of relatively little taxonomic significance at the generic level, *Endodesmidium* ceases to have any features very distinct from *Micromyopsis cristata*. Such considerations lead me to believe that the genus *Endodesmidium* may be superfluous. Nevertheless, the so-called soral membrane in *E. formosum* does not split as in *Micromyopsis*. Tentatively, *Endodesmidium* is retained here but accommodated in the Micromycosidaceae in view of its close similarity to *Micromyopsis cristata*.

*Micromycosidaceae* fam. nov., Chytridiales

Simple chytrids with endobiotic holocarpic thalli, the thallus functioning as a prosorus or a prosperangium, the sorus or sporangium being produced at the tip of a prosoral or prosperangial tube. "Primary" swimmers from sporangia encysting and each cyst producing several "secondary" swimmers. Swimmers posteriorly uniflagellate. Resting stage not known.

Type genus: *Micromyopsis* Scherffel, 1926, *Arch. Protistenk.* 54 : 202. Foundation species: 1. *Micromyopsis cristata* Scherffel, 1926, *Arch. Protistenk.* 54 : 202, Pl. 9, Figs. 65-68; Pl. Figs. 69-76. 2. *Micromyopsis fischerii* Scherffel, 1926, *Arch. Protistenk.* 54 : 208, Pl. 10, Fig. 77. Canter, 1949, *Trans. Br. mycol. Soc.* 32 : 73-77, Text-Figs. 3, 4, Pl. IX, Figs. 1-3.

*Micromycosidaceae* fam. nov., Chytridiales

Chytridae simplices cum thallis endobioticis holocarpicis; thallus agens ut prosorus vel at prosperangium, sorus vel sporangio producto ad apicem tubi prosoralis vel prosperangialis. Greges primarii ex sporangii incystantes et unaquaeque cysta producens plures greges secundarios. Greges postice uniflagellati. Periodus quiescens ignota.

Type genus: *Micromyopsis* Scherffel, 1926, *Arch. Protistenk.* 54 : 202. Species: *M. cristata* Scherffel, *M. fischerii* Scherffel.

The genus *Caulochytrium* Voos & Olive is based on *C. gloeosporii* Voos and Olive<sup>3,4</sup>. *C. gloeosporii* was isolated from dead, attached leguminous pods collected in Miami, Florida, U.S.A. It has been shown to be a eucarpic monocentric chytrid with typical uniflagellate swimmers and a unique life cycle that includes development of distinct haploid thalli whose swimmers may function as zoospores and perpetuate the haploid phase, or function as gametes. The diploid thallus is represented by the zygote resulting from the fusion of isogamous gametes and a stalked aerial zoosporangium that

develops from the zygote. Typically, eight zoospores are formed within the sporangium (meiosporangium), presumably following meiosis so that these zoospores evidently serve to re-establish and perpetuate the haploid phase. *Caulochytrium* cannot be conveniently placed in any of the existing chytrid families and it seems logical to establish a separate family to accommodate it.

*Caulochytriaceae* fam. nov., Chytridiales

Eucarpic monocentric chytrids with separate haploid and diploid thalli. Zoospores posteriorly uniflagellate. Planonts from sporangia of haploid thallus functioning as zoospores and perpetuating the haploid phase, or fusing in pairs to produce a zygote. Diploid thallus represented by zygote giving rise to an aerial sporangium; sporangium typically producing 8 zoospores (following meiotic divisions), the zoospores perpetuating the haploid phase.

Type genus: *Caulochytrium* Voos and Olive, 1968, *Mycologia* 60 : 731. Type species: *C. gloeosporii* Voos & Olive, 1968, *Mycologia* 60 : 731, Figs. 1-5. see also Voos, 1969, *Am. J. Bot.* 56 : 898-909.

*Caulochytriaceae* fam. nov., Chytridiales

Chytridae eucarpicae monocentrales cum thallis haploidis et diploidis. Zoosporae postice uniflagellatae. Planontae ex sporangiis thalli haploidi vel agentes ut zoosporae et perpetuantes phasim haploidicam vel coalescentes binatim ad zygotam producendam. Thallus diploides relatus zygotam producens sporangium aerium; sporangium typice producens 8 zoosporas (secundum divisiones meioticas), zoosporis phasim haploidam perpetuantibus.

Type genus: *Caulochytrium* Voos & Olive, 1968, *Mycologia* 60 : 731. Type species: *C. gloeosporii* Voos & Olive, 1968, *Mycologia* 60 : 731, Figs. 1-5. Voos, 1969, *Am. J. Bot.* 56 : 898-909.

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## PLANTLETS THROUGH SHOOT-TIP CULTURES IN PINEAPPLE

PLANTLET production through *in vitro* culture of shoot tips is now possible in many plant species like carnation<sup>1</sup>, geranium<sup>2</sup>, gladiolus<sup>3</sup>, orchid<sup>4</sup>, tobacco<sup>5</sup> and chrysanthemum<sup>6,7</sup>. Such a technique of meristem culture is not known to have been standardised in pineapple, though the benefits accruing from such a technique are numerous. Firstly, pineapple offers itself as an ideal material for mutation research since the rate of spontaneous mutations is very high in this crop. If one is able to obtain intact plants developing from minimum amount of tissue, this would reduce the chances of diplontic selection in a mutation breeding programme thereby obtaining whole plant mutants than merely obtaining chimeras. In such a system it is also possible to incorporate the growth media with mutagens and then study the mutagenesis. Secondly, such a technique would greatly facilitate fast multiplication of single plant selections in a plant breeding programme in a shorter time as compared to the conventional methods. Thirdly, meristem culture offers an ideal method to obtain virus-free plants which could later form the source of supply of virus-free planting material. The present communication deals with the successful standardisation of such a technique in pineapple.

Young slips of one to one and half month age were used from Kew variety of pineapple for this experiment. The leaves were completely removed from the slips to the extent to which it was possible and tiny growing apex was removed with a size of nearly half an inch. At this stage they were sterilised with 75% alcohol for five minutes and then rinsed in sterile distilled water thoroughly followed by immersion in chlorine water for 5 to 10 minutes. The material was further washed with sterile distilled water and dissected aseptically in the inoculation chamber. During dissection, almost all the scale leaves visible to the naked eye were removed, leaving shoot tip to a very small size measuring about 3–5 mm in size. At this stage the cubed explants were inoculated in different media.

The medium was composed of the same salts as suggested by Knudson<sup>8</sup> for orchid seeds with fortification of micro-elements recommended by Nitsch<sup>9</sup>. Various adjuvants like Indole acetic acid (IAA), Kinetin, Naphthalene acetic acid (NAA) and coconut milk were added to the medium. The media were solidified with 0.9% agar and sterilized by autoclaving at 15 lb/sq. in. for 15 minutes. The pH of the medium was adjusted to 5.8 to 6.0. The cultures were maintained at a temperature of  $26^{\circ} \pm 2^{\circ} \text{C}$  with 12 hour light of 120 f.c. Of the

different media tried, it was observed that the medium with 1 ppm NAA as supplement was the best on which the inoculated meristem developed clusters of thick roots and leaves. At the 4-leaf stage (Fig. 1) the plantlets were subcultured into a medium of same constitution and were allowed to grow under increased light intensity (about 200 f.c.). At the end of a twelve week period these developed into dark green healthy plants with leaves measuring about 30–35 mm in length (Fig. 2). At this stage the plants were removed from the agar medium and planted in pots containing sand and watered with Hoagland's solution. The plants established well and put forth normal new growth.



FIG. 1. One month old seedling.

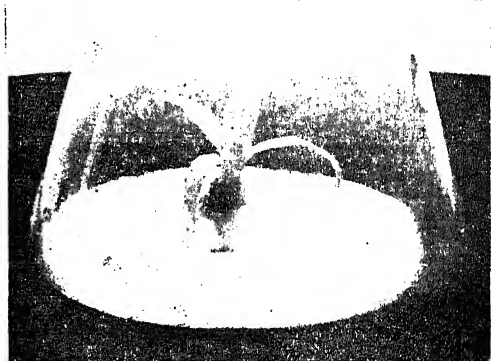


FIG. 2. Three month old seedling.

The results obtained indicate that the growing point from slips of pineapple could be successfully cultured *in vitro* and grown into a full plant. This offers an ideal situation in mutation studies whereby very little of tissues need be treated either with physical or chemical mutagens and yet be able to develop them into full plants where the chances for diplontic selection is limited. This system offers the unique possibilities of (a) inoculation of

mutagen treated tissues in the medium and (b) administration of chemical mutagens to the tissues through the media. The method also ensures that the material is treated at a very early stage of ontogeny and promotes faster growth of treated tissues, which is very essential in the induction of mutation in multicellular buds.

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#### A NOTE ON THE OCCURRENCE OF ABNORMAL FLOWERS IN *GOSSYPIUM* *ARBOREUM* L.

WHILE recording routine observations in the *G. arboreum* breeding materials during 1972-73 season at Regional Agricultural Research Station, Kovilpatti, two abnormal flowers were noticed in one plant of C.C. 1-1-3, a *G. arboreum* variety. The abnormal flowers possessed seven petals as against five in normal flowers. A cross-section of the ovary revealed the presence of seven carpels while it is generally 3 to 5 in normal flowers. The other abnormal flower was allowed to set into a boll. The abnormal boll (Fig. 1) looked like fused bolls and the size and shape were strange and skewed. It had seven small and unequally divided locks totally having 28 seeds.

In order to study the inheritance of this character, all the 28 seeds obtained from this abnormal boll were sown during 1973 season. None of the progenies exhibited any abnormality either in flowers or in bolls. Hence this character

appears to be not heritable. It is probable that some external physical injury at the time of flower initiation might have been a cause for such abnormality.



FIGS. 1-2. Fig. 1. Abnormal boll developed from the multicarpellary (abnormal) flower. Fig. 2. Normal boll developed from a normal flower.

Abnormal flowers with seven petals had formerly been recorded in MCU. 2, a *G. hirsutum* variety (Krishnasamy Rao, 1967)<sup>1</sup> wherein the flower was male sterile. But in the present instance, the development of essential organs of flowers was normal and fully fertile. The setting of boll was not affected and the seed development and number of seeds were similar to that of normal flowers.

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#### EFFECT OF DOWPON ON THE CONTENT OF TOTAL PHENOLICS AND ACTIVITY OF POLYPHENOL OXIDASE IN *CYNODON DACTYLON* PERS.

BEING a selective and translocated herbicide, Dowpon is believed to get accumulated in the subterranean portions in greater concentrations. Flanagan and Langille<sup>1</sup>, reported in Quackgrass that dalapon† ( $\text{CH}_3\text{C}(\text{Cl}_2)\text{COONa}$ ) application resulted in the accumulation of a compound resembling phenol. Four concentrations ( $\text{T}_1\text{-T}_4$ ) of dowpon (Table I) were used; the total phenolics and the polyphenol oxidase activity were

estimated at four intervals of time after application. For total phenolics, the colorimetric method<sup>2</sup> was used and polyphenol oxidase activity was measured by the method suggested by Bateman *et al.*<sup>3</sup>. The total content of phenolics was estimated in the rhizome and the enzyme activity was measured in the foliage.

It was observed that there was a significant change in the total phenolics in the rhizome. The effect of dowpon on the phenolic content in the stolon was prominent, indicating a good relationship between the dowpon treatment and the phenolic content. The phenolic content increased in all the four intervals, namely, 4, 8, 12 and 16 days after spraying the herbicide. In the first and second stages the accumulation was appreciable. Higher phenolic content was recorded in the highest concentration in all the four intervals, viz., 375.00, 313.00 and 230.00% over control (Table I).

TABLE I

*The effect of dowpon on total phenolics content in the stolon of Cynodon dactylon at four stages expressed in mg of pyrogallol/g fresh weight*

Treatment—Dowpon kg/ha No.	Days			
	4	8	12	16
Total phenolics content (mg)				
Stolon				
C—Control ..	0.08	0.15	0.08	0.10
T <sub>1</sub> —5 ..	0.15	0.20	0.15	0.15
T <sub>2</sub> —10 ..	0.24	0.25	0.21	0.15
T <sub>3</sub> —15 ..	0.23	0.28	0.19	0.13
T <sub>4</sub> —20 ..	0.30	0.47	0.26	0.23

The activity of polyphenol oxidase was much inhibited by dowpon. A marked diminishing trend was noticed even in the lowest concentration of dowpon (Table II).

TABLE II

*The effect of dowpon on the polyphenol oxidase activity in the foliage of C. dactylon at four stages expressed as absorbancy/5 min/g fresh weight*

Treatment—Dowpon kg/ha No.	Days			
	4	8	12	16
Polyphenol oxidase activity				
Foliage				
C—Control ..	0.040	0.030	0.030	0.0200
T <sub>1</sub> —5 ..	0.010	0.020	0.030	0.010
T <sub>2</sub> —10 ..	0.010	0.010	0.020	0.015
T <sub>3</sub> —15 ..	0.010	0.010	0.010	0.000
T <sub>4</sub> —20 ..	0.010	0.010	0.010	0.005

Evenari<sup>4</sup> and Mayer and Evenari<sup>5</sup> were of the opinion that the increase in the accumulation of total phenolics is related to dormancy in seeds.

Flanagan and Langilie<sup>1</sup> also observed that "Plant stress is a causative factor in the induction of phenols". Van Overbeek<sup>6</sup> was of the opinion that phenols poisoned the cells and the cells ceased to produce the ATP needed for growth, resulting in the death of the plant.

It is therefore clear that inhibition of the activity of polyphenol oxidase resulted in the high accumulation of phenolic content in the stolon. Even though the activity of the enzyme was measured in the foliage, due to the translocation of the herbicide, the phenolic compounds might have been translocated to the stolons, where a greater accumulation of phenol was seen. Hence the higher phenolic content in treated samples was probably due to the inhibition of the activity of polyphenol oxidase as a result of dowpon action. The phenol was responsible for the injury caused.

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### CERATOCYSTIC SOFT ROT OF SWEET ORANGE

DURING market survey of fruit diseases in Varanasi, a soft rot of sweet orange was noticed in 1971. Affected fruits showed loss of colour and in few cases black patches bearing fruiting bodies over the skin developed. When cut open, numerous black fruiting bodies were found embedded in whitish mycelial mat. The mycelium ramified all over the endocarpic membrane and juicy hairs. In severe cases, the entire fruit turned soft and watery.

The fungus isolated from diseased fruits formed hyaline growth, with a loose cottony appearance on PDA turning light brown in 2 to 8 days finally becoming greenish brown, undersurface turning

dark; conidia produced within 48 hours and mature perithecia within a week; growth intermediate. Aerial hyphae pale brown to hyaline, branched, thin walled, septate and nearly all ends terminating into endoconidiophores,  $2-6\mu$  in width. Submerged hyphae similar except darker and much interwoven. Endoconidiophores pale brown, hyaline at the tip,  $25-125 \times 4-6\mu$ , endoconidia of two types one hyaline, cylindrical, truncate at the ends,  $11-16 \times 4-5\mu$  and the other pale brown to olive brown, barrel-shaped to sub-globose, smooth to rough walled,  $9-16 \times 6-13\mu$ .

Perithecia superficial to immersed, the bases brown to black, globose, sometimes flattened,  $130-200\mu$  in diameter, unornamented or with undifferentiated hyphae attached, neck black, hyaline at the tip, slender upto  $850\mu$  long and  $20-35\mu$  in diameter at the base and  $10-20\mu$  at the tip, ostiolar hyphae hyaline, slender, tapered to a blunt tip,  $8-15$  in number,  $50-90 \times 2-3\mu$ . Asci not seen; ascospores with gelatinous sheath often forming a firm, hat-shaped structure,  $4.5-8 \times 2.5-5.5\mu$ .

The fungus has been identified as *Ceratocystis fimbriata* Ells. & Halst. (Hunt, 1956) and confirmed by the Commonwealth Mycological Institute, Kew (IMI 166925).

When spore suspension of *C. fimbriata* was sprayed on surface sterilized, healthy oranges under aseptic conditions, no symptoms appeared. It seemed that the pathogen enters through wounds or injuries caused during harvesting, transportation, storage or by insects, hence, the injection technique was adopted to test the pathogenicity.  $0.5\text{ ml}$  of spore suspension was injected in each of the six healthy fruits with hypodermic syringe and incubated at  $25^\circ\text{C}$  separately in polythene bags. Equal number of fruits injected with sterilized distilled water were kept as control. After 7-8 days of inoculation, fruits lost their hardness and became pulpy. Soft rot condition developed after 11 days with a rancid odour and complete change in colour while in the control fruits remained healthy. The pathogen was reisolated and was identical with the original isolate.

This is the first report of soft rot of sweet orange incited by *Ceratocystis fimbriata* Ells. & Halst.

The authors express their thanks to Dr. D. L. Hawksworth of C.M.I. for confirming the identification.

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## ENERGY CONSERVING EFFICIENCY OF NATURAL AND MODIFIED GRASSLANDS AT GORAKHPUR

THE efficiency with which solar energy is converted into chemical energy of plant protoplasm, in natural and modified ecosystems is of great importance, since it has a significant bearing in the net primary production, by autotrophs.

Some valuable data are available on the energetics of arctic and temperate countries. But, relatively few are available on the tropical countries. In India, the energy conserving efficiency of the grasslands has been evaluated by Singh and Misra<sup>1</sup>, Gupta<sup>2</sup>, and Mall *et al.*<sup>3</sup>.

The present study deals with the monthly changes in energy conserving efficiency of natural (control) and modified "clipped" stands of *Desmostachya bipinnata* in a typical grassland of Gorakhpur area during the grand growing season (July-October).

The study was carried out during 1973 in two plots of  $20 \times 20\text{ m}$  size, within the campus of Gorakhpur University. Whereas, one plot control (CP) was kept as such, the other (CIP) was modified by "clipping" the above-ground vegetation at mid-height in June, 1973. The net dry matter production was evaluated by "Short term Harvest Method" of Odum<sup>4</sup>. The calorific values were estimated by Oxygen Bomb Calorimeter, and have been expressed in Cal/g dry weight (Table I). The energy captured by *D. bipinnata* was calculated by multiplying the net production with its calorific values. Since data on the interception and albedo were not available for the sites, the efficiency of energy capture was evaluated by expressing the energy captured, as percentages of half the solar radiation received during the period (Table II), which is roughly equivalent to the solar radiation available to plants for photosynthesis (Terrien *et al.*<sup>5</sup>). The data of solar radiation was obtained from the Department of Meteorology, Government of India, Poona. The total solar radiation for the whole year was  $1704160\text{ K Cal/m}^2$  and for the grand growth period (July to October)  $571300\text{ K Cal/m}^2$ .

The data in Table I show that calorific values for above ground parts of *D. bipinnata* range from 3.982 to 4.135 in CP and 4.012 to 4.231 K Cal/g in CIP and for the underground parts from 3.866 to 4.024 in CP and 3.797 to 3.982 K Cal/g in CIP, thus showing higher calorific values in CIP than CP for aboveground parts and in CP than CIP for underground parts. This finding is in conformity with the observation of Wedin<sup>6</sup> and Gupta<sup>2</sup> for the aboveground parts. Further, the energy content for the aboveground parts was

TABLE I

Rate of dry matter production (g/m<sup>2</sup>/month) and energy content (Cal/g) of the aboveground (AG) and underground (UG) parts of *Desmostachya bipinnata* in the control (CP) and clipped (CIP) plots during the grand growth period

Months		Rate of production				Energy content			
		Control		Clipped		Control		Clipped	
		AG	UG	AG	UG	AG	UG	AG	UG
July	..	+100	+164	+186	—	4135	4024	4231	3982
August	..	+162	+215	+265	+184	4049	3989	4159	3941
September	..	+197	+253	+317	±225	4002	3913	4086	3853
October	..	+189	+152	+205	+213	3982	3866	4012	3797

+ Production of dry matter; — Loss of dry matter.

TABLE II

Rate of energy capture (K Cal/g) and % Energy conserving efficiency of the AG and UG parts of *Desmostachya bipinnata* in the CP and CIP during the grand growth period

Months		Solar radiation K Cal/m <sup>2</sup>	Energy captured				% Energy conserving efficiency			
			Control		Clipped		Control		Clipped	
			AG	UG	AG	UG	AG	UG	AG	UG
July	..	149110	413.5	659.9	788.4	—	0.55	0.88	1.06	—
August	..	142290	655.9	857.6	1102.1	525.1	0.92	1.25	1.54	0.73
September	..	140100	788.4	989.9	1295.2	866.9	1.12	1.41	1.84	1.23
October	..	139800	752.6	808.8	822.4	587.6	1.08	1.16	1.25	0.84

higher than that of the underground ones in both the stands. This is in concurrence with the findings of Golley<sup>7,8</sup> for temperate grasslands and Gupta<sup>2</sup> for tropical grasslands.

The data in Table II indicate a contrasting behaviour of the aboveground and underground efficiencies of the two experimental plots. While the clipping enhances the calorific values, net production and efficiency of the aboveground parts ( $t^* = 4.243$ ) it retards them for the underground parts ( $t^* = 3.277$ ). This is probably due to the increase in the aboveground dry matter production, at the expense of the underground parts<sup>1</sup>. Statistical analysis also revealed a positive correlation ( $r = +0.895$ ) between the efficiency and the dry matter production.

The maximum efficiencies by the aboveground and the underground parts were recorded in September (Table II). For the grand growing period the efficiencies were 2.037% in CP and 2.125% in the CIP and for the whole year were

0.68% and 0.70% for the same plots respectively. The values were similar to those obtained by Gupta<sup>2</sup> for the *Dichanthium* community (CP = 0.65%, CIP = 0.98%) at Gyanpur and Asthana (unpublished data) for *Cynodon* community (CP = 0.71%, CIP = 0.84%) at Gorakhpur. Comparatively much lower values (0.33%, 0.35%) were recorded by Singh and Misra<sup>1</sup> for the undisturbed and biotically disturbed grasslands at Varanasi and 0.38% and 0.33% for the natural *Dichanthium* and *Setaria* communities at Ujjain and Ratlam respectively by Mall *et al.*<sup>3</sup>.

The study thus shows that the *Desmostachya* stands in the grasslands of Gorakhpur are highly productive and the productivity can be enhanced by clipping.

The authors are thankful to Prof. K. S. Bhargava, Head of the Botany Department, Gorakhpur University, for providing laboratory and library facilities. Department of Botany, R. SAHAI.  
University of Gorakhpur, M. ASTHANA.  
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March 7, 1974.

\* Significant at 5% level.

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#### A NEW BASE NUMBER FOR THE GENUS *CHEIRANTHUS* L.

SPECIES of the genus *Cheiranthus* (family Cruciferae) are popular garden ornamentals. Except for a doubtful count of 40–42 chromosomes in *Cheiranthus allionii* L., the remaining seven species possess numbers multiple of 7 (Fedorov<sup>2</sup>). The genus, obviously, is monobasic.

*Cheiranthus cheiri* L. has in the past been worked out by Jaretsky<sup>3</sup> (1928), Manton<sup>4</sup> (1932) and Sakai<sup>5</sup> (1935) who report it as a diploid with  $2n = 14$ . While making a chromosome survey of the cultivated plants, two cultivated populations of *Cheiranthus cheiri* L. were found to have  $2n = 12$ . With regard to their behaviour, the plants were quite stable. At prophase the chromosomes pair perfectly and form six bivalents. At metaphase two bivalents are slightly larger in size than the other four (Fig. 1). Most of the bivalents have

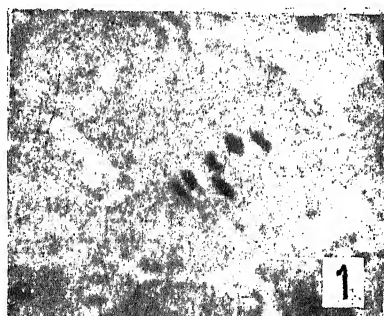


FIG. 1. A pollen mother cell at Metaphase I bearing 6 II  $\times$  6000.

a single chiasma. The distribution of chromosomes at anaphase-I is quite regular. On account of normal meiosis, the plants produce viable pollen and set abundant seed indicating that the plants are amphimicts. This, therefore, creates the possi-

bility of a second base number  $x = 6$  not only for this species but for the genus as a whole.

The tribe Hesperideae to which *Cheiranthus* belongs is polybasic with  $x = 7, 8, 10, 12$  and 13 (Fedorov<sup>2</sup>, Darlington and Wylie<sup>1</sup>). The present count for *Cheiranthus cheiri* is interesting because 6 is the smallest number so far known in the tribe.

In view of the existence of diverse base numbers, aneuploidy seems to have played a major role in evolution within this tribe. Hybridization of the two cytotypes of *Cheiranthus cheiri* should form a very interesting line of investigation as it is likely to reveal the relationship between the two chromosome races.

Authors acknowledge with thanks the encouragement received from Dr. Y. R. Malhotra.

Department of Bio-Sciences, A. K. KOUL.  
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#### RHIZOPUS STOLONIFER, CAUSING SOFT ROT OF SQUASH (*CUCURBITA PEPO* L.)

Soft rot disease caused by *Rhizopus stolonifer* (Fr.) Lind. is severe under storage and transit conditions but it is not true always. Reports are available to support the view of its being a disease causing agent under natural conditions (Singh *et al.*)<sup>4</sup>.

During the course of investigations of diseases of cucurbits in the month of July and August, 1973, a severe rotting of squash fruits (var. *Patty Pan*) was observed at Experimental Farm, Hesaraghatta, Bangalore. The infection may commence from any point of the fruit. The fungus was found to attack on young to mature fruits and flowers. The disease is characterised by water-soaked areas on the surface of the fruit which in turn covers the entire surface and makes the fruit soft. The affected tissues disintegrate and make the flesh soft and pulpy. After about 3–4 days whisker like sporangio-phores develops which bears sporangia on their tips. The mycelial growth is fluffy and continue to grow until entire surface of the fruit is covered. The infected fruits turn completely black and become soft and watery (Fig. 1). The humid weather favours the severity of the disease. Approximately 20–25% of the flowers and fruits were found to be infected under natural conditions.



The fungus was isolated in oat meal agar medium. In culture mycelium was abundant, dirty white in colour with fluffy growth. Sporangia and sporangiophores are observed just after 4 to 5 days of inoculation.

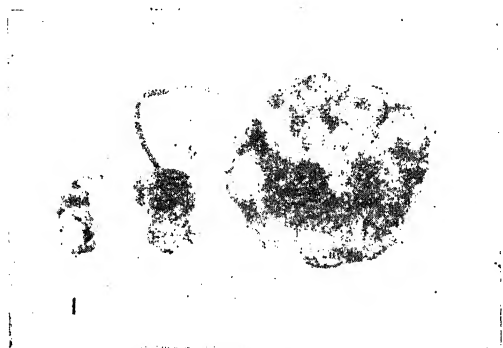


FIG. 1

Pathogenicity test was conducted *in vivo*. Symptoms of fruit rot were observed on the fruits after 15 days of inoculation and resembled that observed in natural condition. Reisolation from the artificially inoculated fruit yielded the same pathogen thereby confirming the pathogenic nature of the fungus.

A search of literature reveals, there is no previous record of this pathogen causing soft rot disease of squash in nature from India or elsewhere. However, Agrios<sup>1</sup>, Anderson<sup>2</sup> and Stevens and Wilcox<sup>3</sup> have reported the fungus causing soft rot under storage and transit conditions. The specimen has been deposited in the Commonwealth Mycological Institute, Kew, Surrey, England (IMI. 179391).

The author is grateful to Dr. G. S. Randhawa, Director, Indian Institute of Horticultural Research, Bangalore, for providing necessary facilities and encouragement. Thanks are also due to Dr. Prem Nath, Sr. Geneticist and Mr. O. P. Dutta for supplying the seed of *Patty Pan* variety of Squash and Dr. Anthony Johnston, Director, C.M.I., England, for confirming the identity of the fungus.

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# ON A NEW SPECIES OF *BALANTIDIUM* CLAPAREDE AND LACHMANN FROM AN INDIAN TORTOISE

THIS paper deals with the description of a new species of *Balantidium* Claparede and Lachmann found in the rectal contents of a tortoise, *Lissemys punctata punctata* (Bonnaterre) purchased from a market of Calcutta, India.

So far, a single species of *Balantidium*, namely, *B. testudinis* Chagas has been reported from turtles although 35 congeneric species have been found to occur in the guts of different vertebrate hosts. The hosts harbouring *B. testudinis* are *Testudo graeca* (type host), *T. radiata*, *T. calcarata* and *Geomyda trijuga*.

*Balantidium dogieli* n. sp. (FIGS. 1 & 2)

FIGS. 1-2. *Balantidium dogieli* n.sp., dorsal view.

**Description.**—It is oval in shape measuring 79.4 (56.3-93.8)  $\mu$ m in length and 55.3 (45-67.5)  $\mu$ m in width. Cilia are long and arranged regularly on

the surface of the body. Peristomeal cilia are longer and thickly grown. Peristome is anteriorly placed with slantingly directed cleft not reaching even up to the anterior third of the body. There is a 'siderophile' lip (Alexeieff, 1931) which apparently possesses a strong affinity for the basic stains. Macronucleus is elongated and located near the middle region of the body measuring 31.4 (22.5–37.5)  $\mu\text{m}$  in length and 6.7 (5.6–7.5)  $\mu\text{m}$  in width. Micronucleus is very small and situated adjacent to the macronucleus. Cytoplasm is alveolar containing numerous food material. Contractile vacuole single and located below the middle region of the body.

The living specimens studied in normal saline showed two types of movement—one was a rapid rotation of the body in dorsoventral plane without any progressive locomotion and other was a progressive spiral movement.

**Type.**—Holotype on slide; paratypes—5 specimens on 3 slides will be deposited in the Zoological Survey of India.

**Comparison.**—Among all the species of *Balantidium* described so far *B. dogieli* n.sp., however, resembles *B. testudinis* only in shape. *B. testudinis*, though lacks any mensural data, is easily distinguishable from the new species under report in having oval macronucleus with a micronucleus embedded in it, longer peristome and in the absence of any contractile vacuole.

The name *Balantidium dogieli* is proposed for this new species after the name of a renowned protozoologist, V. Dogiel.

Authors' sincere thanks are due to the Director, Zoological Survey of India, for the facilities provided in connection with this work.

Zoological Survey ASOK KUMAR DAS.  
of India, RATHINDRA NATH MUKHERJEE.  
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## A NOTE ON INHERITANCE OF BUSHY MUTANT IN CHICKPEA

THE high frequency of spontaneous mutations in Chickpea has often evoked considerable interest of plant breeders and geneticists. A spontaneous macro-mutant in *Cicer arietinum* L. described as 'Bushy mutant' was spotted during 1970–71 in L. 168, a green seeded variety. It has also been reported as dwarf mutant by Athwal, Bhalla, Sandhu and Brar (1970) in the  $X_2$  generation of G 24 with 3500 *r* units. In the present investigation the inheritance of this mutant has been studied.

### MATERIALS AND METHODS

Bushy mutant was crossed with its normal parent, L. 168. Observations on different phenotypes were recorded at appropriate stage of growth in parents,  $F_1$ ,  $F_2$  and backcross generations.

### RESULTS AND DISCUSSION

**Phenotypic expression.**—A brief description of the bushy mutant with its normal parent is given in Table I. Phenotypic expression of this mutant (Fig. 1) shows some resemblance with dwarf mutant reported by Athwal, Bhalla, Sandhu and Brar (1970). However, bushy mutant differs from the dwarf mutant for a number of characters. It has higher number of branches per plant with short internodes, small pod size (Fig. 2). Less number of seeds per pod plate 3 and green seeds.



FIG. 1. Plant growth habit. 1, Bushy mutant; 2, Normal plant.

TABLE I

Comparison of bushy mutant and normal parent for different characters

Characters	Leaf length	Leaflets per leaf	Leaflet size	Plant height	Branches per plant	Pods per plant	Pod size	Seeds per pod	Seed colour	100-seed weight
Bushy mutant	4.2 cm.	13	10×4 mm	20–25 cm	60.4	90.6	Small	1.06	Green	15 gm
Normal parent	5.5 cm.	13	13×7 mm	60–65 cm	29.9	170.8	Medium large	1.35	Green	20 gm

TABLE II

*Sergragation in backcross and  $F_2$  populations of the cross bushy mutant  $\times$  normal*

Generation	Observed		Expected		$X_2$	Remarks
	Normal	Bushy	Normal	Bushy		
$Bc_1$ ( $F_1 \times$ Normal)	40	..	40	..	..	..
$Bc_2$ ( $F_1 \times$ Bushy)	18	17	17.5	17.5	0.003	Good fit for 1 : 1
$F_2$	443	157	450	150	0.435	Good fit for 3 : 1

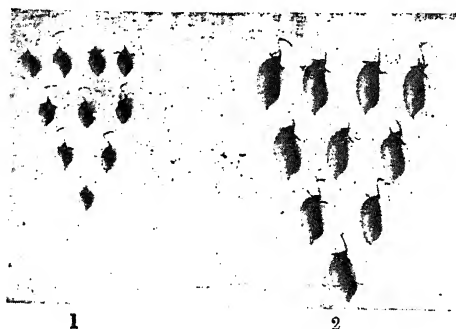


FIG. 2. Comparison of pod size. 1, Bushy mutant; 2, Normal plant.



FIG. 3. Placement of pods on branch and seed per pod. 1, Bushy mutant. 2, Normal plant.

*Inheritance.*—The  $F_1$  of the cross between bushy mutant and normal type was similar to normal

parent thus showing its dominance over bushy mutant type. But  $F_2$  segregated into two phenotypic classes of plant types normal and bushy. The observed classes in  $F_2$  are shown in Table II. The  $F_2$  derived from the cross showed a good fit to the expected ratio of 3 normal : 1 bushy. The monogenic inheritance was also confirmed by segregation ratios in backcross progenies (Table II).

Thus it indicates that the mutant gene controlling plant type affects several seed characteristics also. Two alternative assumptions regarding the nature of this mutant locus are possible. First, this locus pleiotropically controls all these traits studied. Alternatively, this locus might represent a compound locus consisting of more than one closely linked genes subject to rare recombination. As no recombinants of these characters could be detected, the later assumption could not be established and hence the mutant locus can be considered to be pleiotropic in action. A single pair of recessive factors *bs/bs* can be ascribed to this mutant.

## SUMMARY

'Bushy' macro-mutant in chickpea (*Cicer arietinum* L.) was found to affect a number of plant and seed characters. A single pair of recessive factors *bs/bs* was ascribed to this mutant and monogenic inheritance was observed.

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## SHORT SCIENTIFIC NOTES

### Preliminary Observations on Incompatibility in Sweet Potato (*Ipomoea batatas* Poir.)

In flowering plants, self-incompatibility is one of the mechanisms which facilitate outcrossing. This outcrossing device gives opportunity for intervarietal as well as interspecific hybridization and the consequent maintenance and release of variability. It forms a basis for hybrid vigour also. In plant breeding programmes involving self-incompatible individuals hybridization procedure is made easy as it does not require hand emasculation. Cross-incompatibility on the other hand impedes hybridization programmes.

reveal, all the three female parents are self-incompatible. Cross-incompatibility was observed in two combinations, namely, 1.72-30  $\times$  1.72-02 and 1.72-04  $\times$  1.69-16. In the other combinations the percentage of fruitset varied from 1.96 to 71.42.

Hernandez and Miller (1962) reported that compatibility in Sweet potato ranges from zero to 80%. The results of the present study also agree to this observation though the maximum setting is only 71.42%. It was also reported that the compatibility nature of the same variety varies considerably in different combinations both as male and female parents (Togari and Kawahara, 1942; Van Schreven,

TABLE I  
Showing percentage of fruitset

Female parents	Male parents										
	1.67-22	1.67-43	1.68-01	1.68-10	1.68-45	1.69-16	1.72-02	1.72-03	1.72-04	1.72-05	1.72-06
1.69-16	21.42	4.65	64.00	52.94	71.42	S.I.	59.25	39.28	2.50	36.00	25.00
1.72-03	59.90	25.45	23.40	2.04	1.96	50.00	C.I.	S.I.	3.39	2.27	2.01
1.72-04	25.00	8.16	16.66	20.00	33.33	C.I.	8.69	12.50	S.I.	9.80	10.41

S.I. = Self-incompatible, C.I. = Cross-incompatible.

A study of incompatibility in Sweet potato (*Ipomoea batatas* Poir.) varieties was started in the Department of Botany, University of Kerala and preliminary results are presented in this note.

From one hundred and forty varieties of Sweet potato collected from various parts of India and abroad, eleven flowering varieties were selected for the present study. Of these, three profusely flowering varieties were used as female parents. Ten varieties were used as male parents in crosses with each of the three female parents. Fifty flowers were pollinated under each combination.

The flowers from the female parents selected for crossing were emasculated and bagged with butter paper bags between 4 and 5 p.m. Flowers of the male parent were merely bagged. Pollination was done next day morning between 4.30 and 6.30, bagged again and labelled. The bags were removed the same day between 9 and 10 a.m. Seeds from the successful crosses were collected at full maturity, i.e., 20 to 27 days after pollination.

The percentage of fruitset in various combinations used is given in Table I. As data in the table

1953; Hermon, 1960; Wang, 1964). The present findings are also in conformity with this.

Dept. of Botany, P. GOPALAKRISHNA PILLAI,  
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May 17, 1974.

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### A Computer Program for Calculating Alkaline and Acid Phosphatase Activity

Computer programming has found its application in plant breeding and genetics<sup>1-4</sup>. However, its utility in enzymology is not yet known. The purpose of this note is to report a computer program

for calculating the activity of acid and alkaline phosphatase by the method of Bodansky<sup>5</sup>, which is widely employed, with modification to permit the use of Fiske and Subba Row<sup>6</sup> method, for the determination of the phosphate liberated. The program formulated has been successfully employed and it cuts down the time consumed and the cumbersome process of calculating with a desk calculator.

The authors are thankful to the Department of Computer Science, Birla Institute of Technology and Science, Pilani, for the facilities provided.

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#### Occurrence of *Solanum elaeagnifolium* Cav. in Mysore City, Karnataka

During the course of the botanical exploration in Mysore District, some interesting specimens of Solanums were collected, which on identifying keyed out to be *Solanum elaeagnifolium* Cav. The plant is a new record for Karnataka State since the occurrence of this is not mentioned in any of the South Indian floras and monographs. This species is a native of Mexico, and other States of Central America where it is a common weed. The plants were observed in only one or two isolated clumps in open places in Mysore City, and several attempts to locate this plant in other parts of the District were in vain. The introduction and occurrence of this taxon in a distant locality like Mysore City is intriguing and interesting. The nearest species among the South Indian Solanums with which they can be compared is *S. wightii* Nees; the two can be readily separated as indicated below:

Leaves ovate, cordate, softly tawny pubescent on both surfaces; corolla more than 5 cm across; stamens unequal .. .. *S. wightii*.

Leaves linear-lanceolate to narrowly oblong, grey canescent on both surfaces as are the other parts; corolla less than 2.5 cm across; stamens equal .. .. *S. elaeagnifolium*.

*Solanum elaeagnifolium* Cav. *Icon.* 3: 22. t. 243. 1795—Erect, short lived prickly herbs, up to 30 cm tall; plants covered with dense, fine stellate, silvery canescence; stems unarmed, or with a few prickles. Leaves 6–12 × 1.5–3 cm, linear-lanceolate or narrowly oblong, repand-dentate, grey canescent on both surfaces, prickly along midrib, strongly petioled. Flowers few, in cymes; pedicels and peduncles with straight prickles; calyx lobes linear, subulate, up to 1 cm long; corolla violet to blue, 2–2.5 cm across; anthers connivent, 6–8 mm long. Berries round, 10–12 mm across, yellow; seeds lenticular, dark brown.

*English name*: Silver leaf-nettle.

*Flowers*: April-July; *fruits*: August (rarely observed).

Herbarium specimens examined: R. R. Rao 675 (M.G.M.), near Crawford Hall, Mysore City.

*Distribution*: Native of Mexico and Central American States, spreading towards North America; in Mysore rare, with isolated distribution.

*Notes*: Small gregarious plants, growing as compact population of 20–25 plants; fruit setting rare. We have not observed its spread in other localities round about Mysore City since 1969. The plants are conspicuous by their silvery canescence all over.

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#### Comparative Efficacy of *Bacillus thuringiensis* Berliner Formulations Against *Pieris brassicae* Linnaeus

The larvae of the cabbage butterfly, *Pieris brassicae* Linnaeus, cause much damage to crucifers, especially cauliflower and cabbage. Atwal and Singh (1969) reported 74.6 and 64.9% mortality of this pest with carbaryl 0.15% and Thuricide 0.4%, respectively. Usually this pest is controlled with chemicals, which may leave toxic residues on the vegetable crops if the recommended waiting period is not observed. Keeping this in view, the efficacy of 4 formulations of *Bacillus thuringiensis* Berliner, viz., Dipel WP, Thuricide HPSC, Bactospeine and Thuricide 90TS was compared at 3 concentrations (Table I). Both sides of a 9 cm-dia. cauliflower leaf were sprayed on 3rd February 1974 with 1.5 ml of sprayfluid using an atomizer. The sprayed leaf was offered to 8 laboratory-reared larvae in petri-dishes (10 cm-dia.). After 48 hr, untreated fresh leaves were supplied and replaced on alternate days until the last larva in the experiment died. Mortality counts were made 2, 3, 4, 5 and 6 days after offering the treated food. All

TABLE I  
Comparative efficacy of different bacterial preparations against *Pieris brassicae* L.

Pesticide	Dilution (g./litre)	Cumulative mortality percentage (Back transformation of angular means) at different intervals after offering treated food (days)				
		(2)	(3)	(4)	(5)	(6)
Dipel WP (16 10 <sup>3</sup> IU/mg)	0.5	50.0 <sup>bed</sup>	8.9 <sup>b</sup>	100.0	100.0	100.0
	1.0	83.1 <sup>ab</sup>	98.6 <sup>a</sup>	100.0	100.0	100.0
	1.5	88.3 <sup>ab</sup>	100.0 <sup>a</sup>	100.0	100.0	100.0
Thuricide HPSC (16 10 <sup>3</sup> IU/mg)	0.5	85.3 <sup>ab</sup>	100.0 <sup>a</sup>	100.0	100.0	100.0
	1.0	79.5 <sup>ab</sup>	100.0 <sup>a</sup>	100.0	100.0	100.0
	1.5	97.0 <sup>a</sup>	100.0 <sup>a</sup>	100.0	100.0	100.0
Bactospeine (2 10 <sup>3</sup> U.A.A.K. mg)	0.5	6.7 <sup>c</sup>	54.2 <sup>c</sup>	95.2	98.5	100.0
	1.0	20.2 <sup>de</sup>	54.2 <sup>c</sup>	78.8	94.3	100.0
	1.5	20.2 <sup>de</sup>	68.3 <sup>bc</sup>	78.8	93.3	100.0
Thuricide 90 TS (3 10 <sup>3</sup> spores/ml)	0.5	20.5 <sup>de</sup>	50.0 <sup>c</sup>	83.7	98.5	100.0
	1.0	16.3 <sup>de</sup>	41.4 <sup>c</sup>	83.7	93.3	100.0
	1.5	32.9 <sup>cd</sup>	66.8 <sup>bc</sup>	91.6	98.5	100.0
F test		*	*	NS	NS	NS
SE		13.7	7.8	..	..	..

Figures are average of three replication. Means followed by a common letter in a given column do not differ significantly ( $p > 0.05$ ) as per Duncan's multiple range test. IU- International unit, and U.A.A.K. Unit of activity *Anagasta kuehniella*.

the microbial preparations proved significantly superior to control in which there was no kill. Differences between the 12 microbial control treatments were significant only 2 and 3 days after offering of treated food. Thuricide HPSC at 1.5 g/litre and Dipel 1.5 and 1.0 g/litre proved almost equally effective as the differences were non-significant ( $p > 0.05$ ). The differences between the 12 treatments were non-significant 4, 5 and 6 days after offering treated food to the larvae.

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Minerals and Chemical Corporation, California, for supplying Dipel WP, Thuricide HPSC, Bactospeine and Thuricide 90TS respectively.

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## REVIEWS AND NOTICES OF BOOKS

**Pest Control in Groundnuts.** Edited by Susan D. Peakin. Pans Manual No. 2. Third Revised Edition. (Centre for Overseas Pest Research, Pans Office, College House Wrights Lane, London, W 8, SSI, England). Pp. 197. Price £0.50.

This is a completely revised edition of one of the manuals on pest control in tropical crops, produced by the Centre for Overseas Pest Research, London. Groundnut growers will find in this handy manual, a fairly comprehensive source of reliable and practically useful information on various pests that attack the groundnut crop in many tropical and sub-tropical countries. It also deals with diseases, weeds and storage insects of groundnuts.

An introductory section gives a brief account of the distribution, botany, cultivated forms,

agronomy and breeding of groundnut. This is followed by a small section on weeds, which includes two tables summarising recommendations for chemical weed control in groundnut. Various groups of disease organisms, nematodes, insects and mites causing damage to groundnuts in the field are described in the next three sections. There are 90 figures, mostly showing diseased plants or plant parts, insect pests, and distribution maps for the more widely distributed pests and diseases. Chemical control recommendations are given in the form of tables. The problem of Aflatoxin (caused by *Aspergillus flavus*) is discussed in a separate section. Each section has a bibliography of selected references. A checklist of diseases and insects is appended and a subject index provided at the end.

T. SANKARAN.

**Relevant Problems for Chemical Principles** (2nd Edition). By I. S. Butler and A. E. Grosser. (Addison Wesley Pub. Co., Inc., Reading, Mass. 01867, U.S.A.), 1974. Pp. ix + 523. Price not given.

A glance at the University question papers in chemistry in this country would reveal that sufficient attention has not been paid towards the solution of numerical problems. Students can derive elaborate equations in quantum chemistry or thermodynamics but they fight shy of attempting questions involving problems. This tendency is obviously due more to lack of application than any innate difficulty in problem solving, although in some cases, students may not have sufficient mathematical background.

This book has been a very valuable supplement on problems to 'chemical principles' by Dickerson, Gray and Haight (same publishers) and even the titles of the chapters (18) of the two books are identical. This is a great advantage to the student who can readily get the theoretical information needed in solving the problems. Each chapter of the book, under review, has one page of chemical principles and equations concerned with the problems of the chapter for ready reference, to eliminate unnecessary burden on the memory.

The authors have devoted considerable attention to make the problems less abstract and more interesting. In many cases the problems are associated with the techniques employed, often illustrated by figures and the chemical principles needed to understand the problem. This approach makes the working of the problems more interesting and more meaningful. The authors have chosen the problems from a variety of topics, such as space science, medicine, geology, archaeology, biochemistry, engineering and other branches of knowledge to create a feeling of involvement in the student. This approach is a very welcome feature of this book and obviates the feeling of drudgery and aversion towards problems as mathematical jugglery.

Since chemical principles are highlighted along with the problems, the shortest approach towards the solution is not always preferred. The reviewer has gone into the details of the solution of several problems and in many cases, the solutions are rather elaborate. This approach gives greater experience and better knowledge of the fundamentals, to the student. The problems and solutions are grouped separately preventing the inquisi-

tive eye to take a glance at the solutions as he is reading the problem.

At the end of each problem, the correct answer is coupled with three or four incorrect answers to enable the student to know the common mistakes. In the opinion of the reviewer, this is a futile exercise, since a good student need not know how to go wrong as there are many ways of getting wrong answers. An appendix on SI Units is very desirable in a book like this.

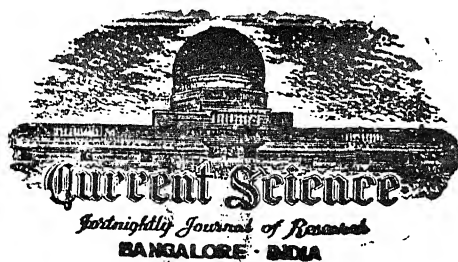
The book is ideally suited for both the undergraduate and graduate courses of Indian Universities. The reviewer unhesitatingly recommends this book both to teachers as well as students specialising in chemistry at the University level.

M. R. A.

**Abies and Picea—Morphological Studies.** By K. A. Chowdhury. Botanical Monograph No. 9, Council of Scientific and Industrial Research, New Delhi, 1974. 24 × 16 cm, i-viii, Pp. 1-46. text-figures 41 (price not mentioned).

This monograph by Professor K. A. Chowdhury, a well-known Wood Anatomist of the Forest Research Institute and Colleges, Dehra Dun, gives in a brief manner, an account of both *Abies* and *Picea* under the following heads: Description and distribution, Indian species, Morphology and Anatomy including root, shoot and leaf, Cones, Embryology including male gametophyte, female gametophyte, fertilisation and embryogeny, Seed, Cytology, Diseases, Economic uses and Fossil history. The succinct accounts for both these genera are given in a clear manner, amply documented by suitable photographs and line drawings which are very good. After providing the key to the Indian species of *Abies* and *Picea*, it is seen that the various species under these two genera are described and invariably in most of the cases the synonyms are also mentioned. Since this portion is related to a taxonomic treatment, it would have been more useful if the original citation with necessary details were provided both to the valid name and the synonyms. A redeeming feature of the monograph is the absence of mistakes and as usual, it maintains the excellent standard of the Publications and Information Directorate of the C.S.I.R. To students of Gymnosperms, this monograph will be very useful and must therefore find a place in all Universities and Research Institutions.

K. SUBRAMANYAM.



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# A TECHNIQUE FOR ACCURATE MEASUREMENT OF SURFACE ANGLES ON CRYSTAL SURFACES

N. J. BAPAT AND R. K. KHARE

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## ABSTRACT

This paper describes a technique for accurate measurement of the angles on the surfaces of single crystals of ferroelectric substances. These angles are formed due to the change in level on the crystal surface caused by the presence of domains in the crystals.

## INTRODUCTION

**T**HE single crystals of ferroelectric substances (like  $\text{BaTiO}_3$ ,  $\text{KNbO}_3$ , etc.), are characterized by the presence of domains in them. Due to these domains (and hence the domain walls) there is a change in level on the crystal surface, which causes the formation of surface angles<sup>1</sup>. The domains are very small in size but can be observed under ordinary microscopes. The angles that are formed are usually of the order of 60 minutes or less and the crystals themselves are very small (only a few millimetres in size) thus making it practically impossible to measure them using a spectrometer. A special technique of Multiple Beam Interferometry<sup>2</sup> is employed to measure these angles with accuracy.

## TECHNIQUE

Tolansky's Multiple Beam Interferometric method consists of the production of multiple beam Fezeau fringes between two surfaces employing a succession of coherent beams. If the multiple beam interference pattern is formed between a flat glass plate and another nearly flat surface (though it may have a complex shape), the fringe pattern is effectively a contour map of the surface microtopography. The fringes are improved in quality if the two surfaces are coated uniformly with a thin reflecting film of silver (allowing about 10 to 15% transmission).

In order to study the microtopography of the surface of a crystal, it is suitably mounted on a glass plate with the help of some adhesive. The crystal and a flat glass plate are coated with silver using vacuum evaporation technique. The crystal plane is adjusted over the glass plate such that it forms an air wedge with the plane of the glass plate. The fringes are observed in reflected light using the green line of mercury (wavelength = 5461 Å).

## TEST OF THE ACCURACY OF SURFACE ANGLE MEASUREMENTS USING THIS TECHNIQUE

In order to test the accuracy of the results of surface angle measurements by this technique, a preliminary experiment was carried out using Fresnel's biprism in the place of the crystal. The

biprism angle was approximately 60 minutes. It was first coated with a thin reflecting film of silver and its angle was accurately determined using a spectrometer. (This could be easily done because the size of the biprism was sufficiently large and it could be easily mounted on the prism table of the spectrometer.) The reflections from the two surfaces forming the biprism angle were utilized for this purpose. Mean of a number of observations was taken and the result (66.0 minutes) was taken to be correct to an accuracy of half a minute.

The biprism was adjusted over the silvered glass plate such that the edge of the biprism touched the plate only at one point (Fig. 1, point E). The

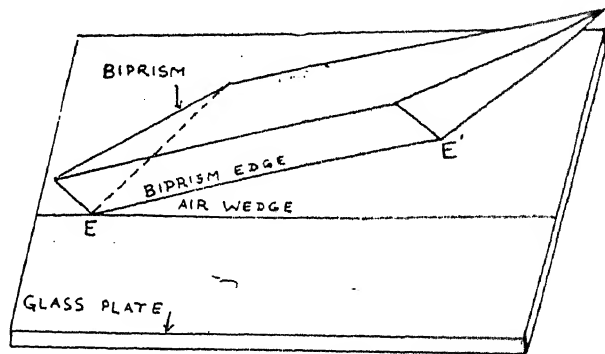


Fig. 1. The arrangement of the biprism over the optical flat to obtain the multiple beam interferometric fringes.

inclinations of the two planes of the biprism were adjusted to be nearly equal on the glass plate. With such an arrangement the interference fringes obtained were as shown in Fig. 2.

If the biprism edge  $EE'$  is parallel to the optical flat, an expected fringe system is a set of equidistant parallel fringes on both sides of the line  $EE'$ .

Figure 3 shows a Multiple Beam Interferogram where the biprism has been adjusted with its edge  $EE'$  parallel to the optical flat. Here it is seen that the fringes near the edge  $EE'$  of the biprism are not equidistant, indicating the rounding off of the edge. Hence while making the measurements for the distance between the fringes, the

situated sufficiently away from the edge EE' were used.

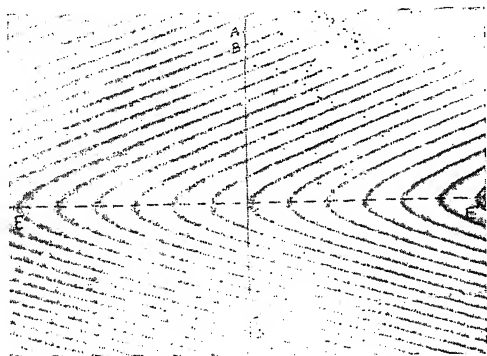


FIG. 2. Multiple Beam Interferometric fringes obtained by the arrangement of Fig. 1 (Magnification 110 X).

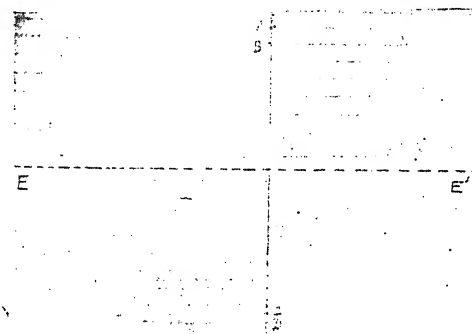


FIG. 3. Multiple Beam Interferometric fringes obtained when the edge EE' of the biprism is parallel to the optical flat (Magnification 115 X).

The biprism angle was calculated from the interferograms of Figs. 2 and 3 using the formula given by Tolansky<sup>2</sup>:

$$\theta = \frac{M\lambda}{2} \left( \frac{1}{AB} + \frac{1}{CD} \right) \text{ radians}$$

where M is the total magnification (this quantity should be accurately found out because the measurements are done on enlarged photographs).

AB and CD are the distances as shown in Figs. 2 and 3. (In practice, the distance between a number of fringes is found out and then it is averaged, in order to minimise the error in the measurements.)

$\lambda$  is the wavelength of the light used.

The value of the biprism angle thus calculated was found to be 66.2 minutes. The spectrometer also gave very nearly the same result thus confirming the accuracy in the use of Multiple Beam Interferometry for such work.

#### SURFACE ANGLES DUE TO DOMAINS IN $\text{KNbO}_3$ SINGLE CRYSTALS

Figure 4 shows the micrograph of the surface of a silvered  $\text{KNbO}_3$  single crystal in reflected light. Figure 5 is the corresponding interferogram over the same surface. The micrograph of Fig. 4



FIG. 4. Micrograph over the surface of the  $\text{KNbO}_3$  single crystal (Magnification 135 X).

reveals a large number of domain walls and the fringes in the corresponding interferogram of Fig. 5

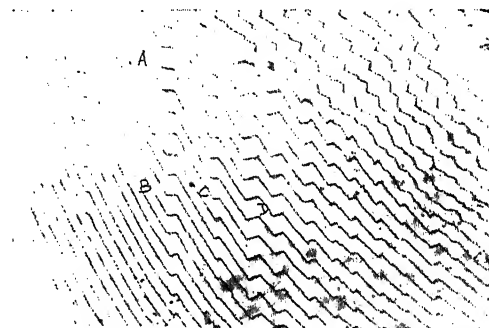


FIG. 5. Corresponding interferogram over the same surface of  $\text{KNbO}_3$  single crystal (Magnification 135 X).

show that there is a definite change in level at each of the domain wall. If the crystal surface were a perfect plane, the interferogram would have shown straight parallel fringes.

The surface angles have been measured at the places marked A, B, C, and D and the values calculated using the formulae of Tolansky<sup>2</sup> are 56.5, 56.6, 56.8, and 57.2 minutes respectively. These values show a close agreement with the theoretical value of 57 minutes, the angle at which the planes across 60° domain walls in  $\text{KNbO}_3$  single crystals are inclined.

1. Bhide, V. G. and Bapat, N. J., *Physica*, 1961, 27, 531.
2. Tolansky, S., *Multiple Beam Interferometry of Surfaces and Films*, Clarendon Press, Oxford, England, 1948.

# THERMAL NEUTRON CAPTURE GAMMA RAYS FROM $^{141}\text{Pr}(n, \gamma)^{142}\text{Pr}$ REACTION

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## ABSTRACT

Thermal neutron capture gamma rays from the product nucleus  $^{142}\text{Pr}$  were investigated in the 0 ~ 900 keV range with a scintillation spectrometer coupled to a 400 channel analyzer at the 'CIRUS' reactor of Bhabha Atomic Research Centre, India. The energies and relative intensities of the observed gamma components were estimated and discussed.

THE nucleus  $^{142}_{59}\text{Pr}_{83}$  is an odd-odd isotope with nine protons and one neutron outside the doubly magic  $^{132}\text{Sn}$  core. The levels excited in such nuclides may be described in the framework of the nuclear shell model and the residual interaction between the last proton and the last neutron. The level structure of  $^{142}\text{Pr}$  can be known only from reaction spectroscopy since the ground states of both the neighbouring isobars  $^{142}\text{Ce}$  and  $^{142}\text{Nd}$  are stable.

The present work on the  $^{141}\text{Pr}(n, \gamma)^{142}\text{Pr}$  reaction was carried out at the 'CIRUS' reactor of Bhabha Atomic Research Centre, Bombay. The energies and relative intensities of gamma rays (in the range 0 ~ 900 keV) were computed using a scintillation spectrometer and a 400 channel analyzer. The experimental technique comprising the reactor shielding arrangement, the target assembly and the electronic instrumentation were described in the earlier works<sup>1,2</sup>. The standardization, data collection and method of analysis were also given in the same references.

## EXPERIMENTATION AND RESULTS

For the present investigation specpure praseodymium in the powdered form of  $\text{Pr}_6\text{O}_{11}$  was obtained from the Pure Material Section of the Chemistry Division of Bhabha Atomic Research Centre. The target was prepared in the form of a cylinder of dia 6 mm and length 12 mm. The  $n\sigma$  value of the same corresponds to 0.109 (Radiative capture cross-section of praseodymium is 11.2 barns). Thermal neutron flux at the target position was estimated to be of the order of  $10^{16}$  neutrons/cm<sup>2</sup>/sec, by Gold foil irradiation method.

The gamma ray detection was accomplished by a  $38 \times 38$  mm NaI(Tl) crystal and the spectrum was scanned with the aid of a 400 channel analyzer. The observed gamma spectrum is shown in Fig. 1. There are eleven lines including the one due to annihilation quanta (A) at 511 keV. The energies of different gamma components are estimated and given in Table I. To determine the relative intensities of the different transitions, the peeling off technique was employed. Corrections due to

self-absorption in the target, photopeak efficiency and absorption in  $^6\text{Li}_2\text{Co}_3$  screen that protected

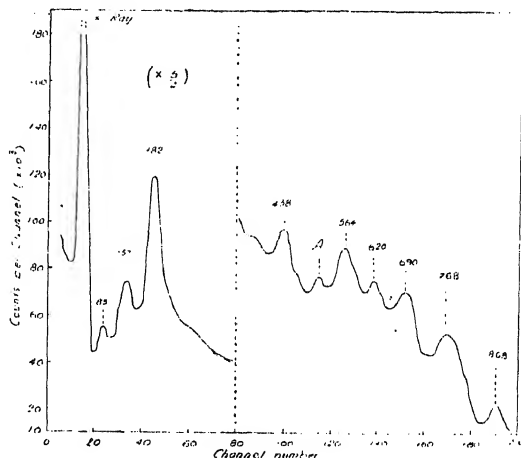


FIG. 1. Capture gamma ray spectrum from  $^{142}\text{Pr}$  (below ~ 900 keV).

TABLE I

Low energy capture gamma rays from  $^{142}\text{Pr}$   
(A comparative data due to other authors  
with similar experimental systems as  
in the present work)

Present work		Giannini <i>et al.</i> <sup>3</sup>	
$E_\gamma$ (keV)	$I_\gamma$ (rel)	$E_\gamma$ (keV)	$I_\gamma$
85 (3)	32 (4)	94 (2)	3
137 (3)	46 (4)	145 (3)	14
182 (3)	100 (5)	177 (3)	18
438m	16 (2)		
511 (4)A	23 (3)		
564m	60 (4)		
620 (5)	62 (5)		
690m	78 (5)		
768m	89 (7)		
868 (7)	40 (5)		

'm' indicates multiplet. The figures in brackets for  $E_\gamma$  and  $I_\gamma$  (rel) represent the errors for the energy and intensity determinations.

the detector from neutron irradiation were computed and applied to the observed gamma intensities. The 182 keV transition was found to be the strongest one and intensities of the other components were expressed relative to this. These results are summarized in Table I. For a comparison, the results of Giannini *et al.*<sup>3</sup>, who used a similar experimental method for low energy gamma rays, are also included in the same table. However, the region of interest in ref. (3) confines only to a few transitions. Some of the lines observed in this work and interpreted as multiplets are indicated by 'm' in Table I. The present measurements are in accordance with those due to the other experimental methods<sup>4</sup>.

### DISCUSSION

The ground state spin<sup>5</sup> of the target nucleus <sup>141</sup>Pr is 5/2<sup>+</sup> while that of the product nucleus<sup>6</sup> is 2<sup>-</sup>. The capture of a *s*-wave neutron will result in the formation of the initial state of <sup>142</sup>Pr with a spin-parity of either 3<sup>+</sup> or 2<sup>+</sup>. In this situation, primary gamma rays of pure E1 type are expected to populate the low lying levels due to the decay of the capture state in <sup>142</sup><sub>59</sub>Pr<sub>83</sub>. The 83rd neutron has a spin-parity 7/2<sup>-</sup> which in the shell model classification is characterised by the ( $\nu f_{7/2}$ ) configuration. The odd proton (59th) may be expected to occupy the ( $\pi d_{5/2}$ ) configuration. In the shell

model description, one expects a formation of six states from ( $\pi d_{5/2}$  /  $\nu f_{7/2}$ ) configuration mixing with spins 1 to 6 and negative parities. From the energy systematics of odd-odd nuclei<sup>6</sup>, the present gamma components with energies 85, 137 and 182 keV may be regarded as those due to the transitions taking place between the corresponding states and the ground state of <sup>142</sup>Pr. The other components may be ascribed to the transitions taking place in the multiplets formed by configuration mixing of orbitals describing the ground and first excited states.

### ACKNOWLEDGEMENT

The authors convey their thanks to Prof. R. Ramanna and Dr. P. K. Iyengar for giving the reactor facility to carry out the present investigation.

1. Chintalpudi, Surya N., Sastry, D. L. and Swami Jnanananda, *Indian J. Pure Appl. Phys.*, 1969, 7, 542.
2. —, — and —, *Il Nuovo Cimento*, 1969, 63 B, 447.
3. Giannini, M., Pinto, G., Prosperi, D. and Sciuti, S., *Ibid.*, 1963, 29, 977.
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5. *Nuclear Data Tables*, Academic Press, 1969, 5 A, 9.
6. Brennan, M. H. and Shugart, H. H., *Phys. Rev.*, 1962, 128, 1796.

## NIOBIUM(V) COMPLEXES WITH AROMATIC SCHIFF BASES

N. S. BIRADAR\* AND T. R. GOUDAR

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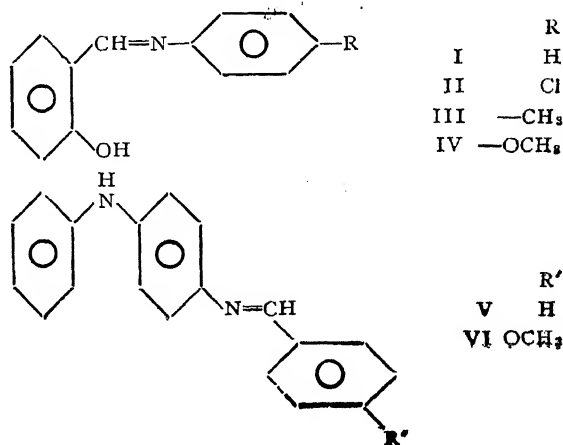
### ABSTRACT

Six niobium(V) complexes with aromatic Schiff bases have been prepared in chloroform and characterised by elemental analysis. These are non-electrolytes in DMF. Infrared spectra have been reported and all the complexes are regarded to have coordination a number of seven.

### INTRODUCTION

THE complexes of niobium(V) with wide range of Schiff bases containing a variety of donor sites have been reported in the literature<sup>1-4</sup>. They have shown that in almost all the complexes the coordination number of niobium is seven. Prashar and Tandon<sup>5</sup> have recently reported hexa- and octa-coordinate Schiff base complexes of niobium(V) and tantalum(V).

This report concerns the synthesis and spectral studies of niobium(V) complexes with the following Schiff bases,



\* To whom all the correspondence should be addressed.

- (i) Salicylidine-aniline.
- (ii) Salicylidine-*p*-chloroaniline.
- (iii) Salicylidene-*p*-toluidine.
- (iv) Salicylidene-*p*-anisidine.
- (v) Benzylidene-*p*-aminodiphenylamine.
- (vi) Anisylidene-*p*-aminodiphenylamine.

#### EXPERIMENTAL

Niobium(V) chloride was of Fluka grade and the remaining chemicals used for the reactions were of reagent grade. The commercial chloroform was dried over fuming sulphuric acid and distilled before use. The Schiff bases were prepared according to the method reported elsewhere<sup>6</sup>.

Niobium pentachloride (0.01 mole) in chloroform was treated with the Schiff base (0.022 mole) in the same solvent with vigorous shaking, and allowed to stand for several hours. The precipitated complex was filtered, washed with chloroform and dried in vacuum over phosphorus pentoxide.

The dry mass was powdered and extracted with dry ether in a soxhlet apparatus and dried at 50–60°C. All the preparations were carried out in a dry box in the absence of moisture.

#### ANALYSIS

Niobium in the complexes was determined gravimetrically as Nb<sub>2</sub>O<sub>5</sub>, chloride as AgCl, and nitrogen by the Kjeldhal method. The analyses are given in Table I.

TABLE I  
Elemental analysis of niobium (V) Schiff base complexes

Lig. No.	Compound No.	Empirical formula	% Nb		% Cl		% N		Colour
			Found	Calc.	Found	Calc.	Found	Calc.	
1	2	3	4	5	6	7	8	9	10
I	VII	(C <sub>13</sub> H <sub>10</sub> ON) <sub>2</sub> NbCl <sub>3</sub>	15.82	15.74	17.98	18.13	4.76	4.75	Yellow
II	VIII	(C <sub>13</sub> H <sub>9</sub> ONCl) <sub>2</sub> NbCl <sub>3</sub>	14.15	14.31	16.17	16.21	4.23	4.24	„
III	IX	(C <sub>14</sub> H <sub>12</sub> ON) <sub>2</sub> NbCl <sub>3</sub>	15.15	14.99	17.13	17.26	4.49	4.51	„
IV	X	(C <sub>14</sub> H <sub>12</sub> O <sub>2</sub> N) <sub>2</sub> NbCl <sub>3</sub>	14.51	14.38	16.26	16.42	4.38	4.30	„
V	XI	(C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> ) <sub>2</sub> NbCl <sub>5</sub>	11.61	11.45	20.38	20.58	6.79	6.89	Violet
VI	XII	(C <sub>20</sub> H <sub>16</sub> ON <sub>2</sub> ) <sub>2</sub> NbCl <sub>5</sub>	10.83	10.63	19.96	20.03	6.31	6.41	„

#### PHYSICOCHEMICAL MEASUREMENTS

The molar conductance was measured with an ELICO conductivity bridge type CM-32, with a cell constant of 0.829 cm<sup>-1</sup>. The infrared spectra of the ligands and the complexes, in nujol mull, were recorded on a Beckman IR-20 recording spectrometer in the region 4000–600 cm<sup>-1</sup>.

#### RESULTS AND DISCUSSION

The analytical data given in Table I show that all the six Schiff bases form complexes of 1:2

stoichiometry with niobium(V) chloride. The molar conductance values, in DMF at the concentration 10<sup>-3</sup> M fall in the range of 1.0–5.0 ohm<sup>-1</sup> cm<sup>2</sup>/mole. These values are too low to account for the behaviour of the complexes as electrolytes; the complexes can therefore be considered as non-electrolytes in DMF.

#### INFRARED SPECTRA

The important infrared frequencies and their tentative assignments are given in Table II. A strong band found in the region 1620–1590 cm<sup>-1</sup>, assignable to the C=N stretch<sup>7</sup> of the bases, is observed in the complexes in the region 1650–1610 cm<sup>-1</sup>. The observed shift obtained in the C=N stretch after complexation suggests that azomethine nitrogen is coordinated to the metal ion. The broad, weak band in the region 2800–2600 cm<sup>-1</sup>, assignable to the intramolecular hydrogen bonded –OH<sup>8</sup> in the bases I–IV, is not found in the complexes. The absence of this band in benzylidene-*p*-aminodiphenyl and anisylidene-*p*-aminodiphenylamine confirms this assignment. The strong band in the region 1280–1250 cm<sup>-1</sup> of the bases I–IV, due to the phenolic C–O, is found in the region 1335–1315 cm<sup>-1</sup> in the complexes. This is indicative of niobium-oxygen bond formation with oxygen of the *o*-OH group of the bases. The infrared spectrum of bases V and VI show a band around 3300 cm<sup>-1</sup> which is attributed to the –NH stretch of the secondary amine group. This band

does not show any splitting in the complexes XI and XII, indicating that the –NH group has not taken part in the coordinate bond formation (Table II).

All these observations lead to the following conclusions.

1. In all the complexes (VII–XII), the azomethine nitrogen has taken part in the coordinate bond formation. As a result of this the bond order of carbon to nitrogen link is increased.

TABLE II

Infrared frequencies (in  $\text{cm}^{-1}$ ) of Schiff bases, niobium (V) complexes and their assignments

I	VII	II	VIII	III	IX	IV	X	V	XI	VI	XII	Assignments
2994	2950	2959	2950	2985	2930	2985	2930	2905	2950	2910	2950	=CH and aromatic CH stretching
2632	..	2703	..	2632	..	2632	..	..	..	..	..	Intra molecular H-bonded OH
1613	1645	1603	1630	1616	1650	1616	1640	1598	1610	1608	1650	C=N stretching
1587	1585	1582	1590	1592	1610	1600	1590	1600	..	1510	..	Aromatic C=C stretching
1567	1525	1563	1530	1567	1550	1564	1550	1575	1550	1480	1550	
1481	1455	1475	1435	1495	..	1488	1450	1497	..	..	1490	
1278	1335	1266	1315	1279	1325	1272	1320	..	..	..	..	Phenolic C—O stretching
..	..	..	..	..	..	..	..	3340	3330	3300	3280	—NH stretching

- Disappearance of hydrogen bonded —OH in the complexes (VII–X) and high frequency shift of the phenolic C—O stretch are the suggestive of niobium-oxygen bond formation.
- Non-splitting of —NH stretch in the complexes XI and XII indicates that —NH group of the bases V and VI has not taken part in the complex formation.

## STEREOCHEMISTRY

Niobium(V) chloride forms complexes of 1:2 stoichiometry with bases I–IV losing two of the five chlorides whereas it forms 1:2 adducts with bases V and VI (Table I). All these complexes are non-electrolytes in DMF. These observations taken together with spectral observations suggest that niobium(V) has coordination number seven in these complexes.

For coordination number seven, there is no simple arrangement of the ligands such that all the nearest neighbours are at equal distances. Nyholm and coworkers<sup>9</sup> have given various stereochemical arrangements for coordination number seven. Amongst them, capped trigonal prism (1:4:2 stereochemistry) is supposed to be the most favoured configuration<sup>10</sup>. With the existing data the stereochemistry of these complexes is not clear. By analogy with diarsine complexes<sup>9</sup> and the prevailing majority of "1:4:2" stereochemistry, it seems possible that these complexes may favour "1:4:2" stereochemistry.

## ACKNOWLEDGEMENT

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# IMAGE RECONSTRUCTION USING FRACTIONAL FOURIER TRANSFORMS

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## ABSTRACT

A method is proposed for a consistent analysis of many 'images' of an object in any plane of observation between the diffraction plane and gaussian image plane of an image forming system, after Wiener-Condon-Patterson's approaches of expressing a Fourier transform as a subgroup of a continuous transformation group.

**T**HE knowledge of the mechanism of image formation and the methods of their reconstruction has been enriched during the last decade. However, some problems are still to be solved, especially in the field of electron microscopy. Image reconstruction methods use many manipulations in the reciprocal or direct space<sup>1,2</sup>. The image itself is then synthesised in a digital or analog computer<sup>3</sup>. Due to a number of optical and phase problems the reconstructed image may not represent those features recorded in the original specimen or micrograph<sup>4</sup>. In electron microscopic objective aperture limited, imaging of objects of normal thickness and even with thin objects with paracrystalline or short range order, it is known that defocused and gaussian focussed images are complicated by the simultaneous existence of positive and negative phase contrasts as well as diffraction contrast<sup>5-7</sup>. In practical electron microscopy, there is a tendency to underfocus the image to within certain limits which will allow contrast to be enhanced by phase contrast. Such defocusing results in artefacts and contrast reversals in the final image<sup>8</sup>. General theory of image formation in optical instruments also raises some doubts whether the electron micrographs showing fine details and fringes representing lattice planes in crystals represent true images<sup>9</sup>. 'Image' off the focal plane equals the Fresnel image of a lensless aperture of suitable size<sup>10</sup>. Such patterns are believed to arise from Fresnel diffraction. For the defocus  $\Delta f > 100$  nm Fresnel diffraction is a dominant feature of the image. However, Fresnel diffraction is usually neglected in the theory of image formation. One wonders whether this readily visible but normally discarded data could not be used for scientific purposes. If it is so, their rationalization could considerably extend the use of image reconstruction.

According to Abbe theory of image formation<sup>11</sup>, if a cross-grating is located to the left of the first focal plane of a convergent lens and illuminated by a parallel beam of light then the diffraction pattern or the Fourier transform of the grating will be imaged in the second focal plane while the grating itself will be imaged in the gaussian image plane to the right of the second focal plane and is considered to be the Fourier transform of the diffraction pattern. It is apparent that the diffraction pattern, gaussian image and the 'image' intensity

distribution in any plane between the focal plane and the gaussian image plane essentially contain the same information, the difference being the manner in which this information is displayed. The problem of expressing the 'image' intensity in any plane between the focal plane and the gaussian image plane as a transform of the intensity in any other such plane requires a solution in terms of Wiener<sup>12</sup>, Condon<sup>13</sup> and Patterson<sup>14</sup> continuous transforms. The operation of Fourier transform generates a cyclic group of order 4 which is isomorphic with the group of rotations of a plane about a fixed point through integral multiples of a right angle<sup>13</sup>. Wiener-Condon-Patterson formulation provides a solution to the problem of embedding the Fourier transform group of order 4 in a continuous group, and a general expression for the kernels of integral transforms which leads to sets of functional spaces lying between any given function space and its Fourier transform space. One may thus visualize fraction Fourier transforms and fractional spaces, ordinary Fourier transform and reciprocal space being particular cases.

Many investigators<sup>15-18</sup> have described procedures in which a series of electron micrographs at different foci are taken and evaluated or image is reconstructed by a combined analysis of gaussian image and diffraction pattern. Here a theoretical discussion is given of some of the possibilities for image reconstruction and evaluation from out of focus images. The principal feature of the method, which is based on fractional Fourier analysis of images, is that the information lost in one image can be replaced by information on other images with different defocusing parameters. This also provides the possibility of a combined phase and diffraction contrast analysis of images, thereby leading to better resolved images or removing the ambiguities in the reconstructed images. After Patterson<sup>14</sup>, the generalized transform may be expressed as:

$$G(\vec{u}, m_1) = \int K(\vec{u}, \vec{x}, dm) \cdot F(\vec{x}, m_2) \cdot d\vec{x} \quad (1)$$

where

$$\begin{aligned} K(\vec{u}, \vec{x}, dm) &= (Q^{1/2} \alpha / (\pi(1-t^2))^{1/2}) \cdot \exp \alpha^2 [(2t/(1-t^2)) \\ &\times (\vec{u} \cdot \vec{x}) - ((1+t^2)/2(1-t^2)) \\ &\times (\vec{x} \cdot \vec{x} + \vec{u} \cdot \vec{u})] \end{aligned} \quad (2)$$



and

$$\begin{aligned} a &= \sqrt{2\pi}(1-\beta^2)^{1/4}; \quad Q = \sqrt{1+\beta^2}-\beta^2; \\ dm &= m_1 - m_2; \quad t = Q \cdot i^{dm} = Q \cdot \exp(i\phi); \\ \phi &= dm\pi/2; \quad \beta \rightarrow 0. \end{aligned} \quad (3)$$

$m_1$  and  $m_2$  are parameters corresponding to various observation planes. The kernel  $K$ , in the limit  $\beta \rightarrow 0$ , approaches a delta function for  $dm = 0$  and 2 and Fourier transform pairs for  $dm = 1$  and 3 and generalized inverse pairs when  $m_1 + m_2 = 4$ . Fractional values between 1 and 2 for  $dm$  corresponds to space transforms between diffraction and image planes.

A simple scheme for image correction and reconstruction will require at least two defocused images, say  $G$ 's, corresponding to the observation planes  $m_1$  and  $m_2$ , related by

$$G(\vec{x}, m_1) = \int G(\vec{u}, m_2) \cdot K(\vec{u}, \vec{x}, dm) \cdot d\vec{u} \quad (4)$$

which can be solved for  $dm$  numerically. Final reconstructed image should be consistent with the following relations, representing 'images' in other observation planes,

$$\begin{aligned} F(\vec{x}, 0) &= \int G(\vec{u}, m_1) \cdot K(\vec{u}, \vec{x}, m_1) \cdot d\vec{u} \\ &= \int G(\vec{v}, m_2) \cdot K(\vec{v}, \vec{x}, m_2) \cdot d\vec{v} \\ &= \int G(\vec{v}, m_2) \cdot K(\vec{v}, \vec{u}, dm) \cdot d\vec{v} \end{aligned} \quad (5)$$

An estimate for  $m$ 's can be obtained from the defocus relation

$$df = \tan \phi \quad (6)$$

Kernel of transformation for certain ranges and values of  $m$  or  $\phi$  can be easily shown to represent the formation of Fresnel and Fourier images<sup>19-20</sup>. Under such conditions Kernel  $K$  is formally equivalent to the kernel for Fresnel transform of the given function, a limiting case of which is the Fourier transform. Fresnel transform is also an unambiguous representation of the function and inversion reconstructs the original function, however, it rarely resembles the original function<sup>21-22</sup>. Although there are some problems with the behaviour of  $K$  for very small values of  $dm$ <sup>14</sup>, the existence of Fresnel transform is assured if the Fourier transform of the function exists. In the range of our interest ( $1 \leq m \leq 2$ ) and somewhat larger values of  $dm$ , initial computations to explore the feasibility of the method, with one dimensional functions, have been carried out, the details of which will be published elsewhere. Here it should be mentioned that the present approach provides a unified view of defocused images and images with wide focal range can be interpreted in a systematic way. Similar to the work of Gerchberg and Saxton<sup>23-24</sup>, who have studied the relationships implied by Fourier theorem between the wave amplitudes and the wave phases at the back-

focal plane of the objective lens and image plane, by the present method the complete wave function in the diffraction plane may be determined from a consistent analysis of the observed intensity distribution of the two or more defocused images. Such fractional images are in general complex<sup>25</sup> but in certain cases are useful in phase recovery<sup>26</sup>. Many complex patterns observed with convergent electron beams<sup>27</sup> may contain such images. The explanation suggested above for the appearance of fractional images and their analysis for reconstructing images is a tentative one, and requires further detailed study, the basic phenomenon is, however, readily understood. Attempts are being made to apply the method to the reconstruction of images from electron microscope.

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## LETTERS TO THE EDITOR

2'-DEOXYADENOSINE-5'-MONOPHOSPHORIC  
ACID DISODIUM SALT

OUR recent X-ray analysis of the disodium salt of 2'-deoxyguanosine-5'-monophosphoric acid (dGMP- $\text{Na}_2$ ) has revealed two unique features—a *gauche-trans* (gt) conformation for the phosphate group about the exocyclic C (4')-C (5') bond and an *endo* puckering of the O (1') atom of the deoxyribose sugar<sup>1</sup>—not found in the crystal structures of other DNA monomers, dAMP<sup>2</sup>, dCMP<sup>3</sup> and dTMP<sup>4</sup>. It was felt that the presence of two Na ions per molecule in the crystal structure could be one of the factors contributing to these special features. An attempt was, therefore, made to crystallise disodium salts of other DNA monomer units. We report below the crystal data of the disodium salt of 2'-deoxyadenosine-5'-monophosphate.

Long (15–20 mm) platy crystals of pedial symmetry were grown by slow diffusion of acetone into water solutions of the sample obtained from Sigma Chemical Company. The crystals develop prominently the prismatic (110) and pinacoidal (100) faces. X-ray and optical goniometric studies show that the crystals are contact twinned, the twin and composition-plane being the (010) plane. The interfacial angles measured on the optical goniometer are:  $\angle 1'1'0' \wedge 1'0'0' = 97^\circ 36'$ ,  $\angle 1'0'0' \wedge 3'1'0' = 51^\circ 47'$ ,  $\angle 3'1'0' \wedge \bar{2}10 = 19^\circ 28'$ ,  $\angle \bar{2}10 \wedge 110 = 10^\circ 19'$ . Different conditions of crystallisation involving changes in buffers and pH values failed to undo the twinning. Rotation, Weissenberg and precession photographs were used in obtaining the unit cell dimensions. The crystal belongs to the triclinic system with two molecules in the unit-cell. The density measurement using carbon tetrachloride and bromoform is consistent with seven water molecules per sample molecule in the unit cell.

*The crystal data of 2'-deoxyadenosine  
5'-monophosphate disodium salt*

Chemical formula	$\text{C}_{10}\text{N}_5\text{H}_{12}\text{O}_8\text{Na}_2\text{P} \cdot 7\text{H}_2\text{O}$
Molecular weight	501.14
System	Triclinic
<i>a</i>	22.66 Å
<i>b</i>	6.98 Å
<i>c</i>	6.84 Å
$\alpha$	96° 56'
$\beta$	93° 21'
$\gamma$	78° 30'
$d_{\text{obs}}$	1.608 gm/cc
$d_{\text{cal}}$	1.607 gm/cc
Space group	$P1$

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\* Primed indices indicate planes from second crystal in the twinned crystal.

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EMISSION SPECTRA OF ISOMERIC  
PYRIDINE-ALDEHYDES

EMISSION spectra of isomeric 2-, 3- and 4-pyridine-aldehydes have been studied at room temperature as solution in various solvents. It has also been studied at liquid nitrogen temperature in solid solution on Aminco-Bowman Spectrophotofluorometer. It was observed that all the three isomers do not give any emission either in hydrocarbon or hydroxylic solvents at room temperatures. At liquid nitrogen temperatures all the three isomers give emission and the wavelength of emission maxima and excitation maxima are given in Table I.

TABLE I  
*Phosphorescence in pyridine-aldehydes in  
ethanol at 77° K*

Compound	Phosphorescence maxima $m\mu$	Excitation maxima $m\mu$
2-pyridine-aldehyde ..	415	320
3-pyridine-aldehyde ..	430	325
4-pyridine-aldehyde ..	440	330

That the emission is due to phosphorescence has been proved by recording the same band by using a phosphoroscope. The singlet-singlet absorption studies showed that the lowest singlet in all these isomers is of the  $n-\pi^*$  type, and since the quantum yield is fairly high, the lowest triplet state giving rise to this phosphorescence emission is a  $^3(n\pi^*)$  state. Comparison of the results with similar studies on other pyridine derivatives<sup>1,2</sup> namely,  $\text{NH}_2$  and CN, confirms this assignment.

In pyridine aldehydes, there are two types of  $n-\pi^*$  transitions, namely, those involving ring nitrogen lone pair and the aldehydic carbonyl lone pair.

This makes the study of these compounds interesting. By comparing the vibrational assignments in pyridine-aldehydes and benzaldehyde, it has been shown that the  $^3(n\pi^*)$  in case of pyridine aldehydes arises from ring nitrogen and not the one corresponding to aldehydic group.

Detailed paper will be published elsewhere.

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### FREE ENERGY OF ADSORPTION OF BENZOTRIAZOLE ON COPPER SINGLE CRYSTAL PLANES IN SULPHURIC ACID

BENZOTRIAZOLE\* (BTA) is an effective corrosion inhibitor of copper<sup>1-5</sup>. Copper is an industrially important metal next to iron and the study of inhibition of corrosion of copper in acid solution is a subject with wide technological significance. In the previous literature<sup>6</sup> on electrochemical process, different forms of adsorption isotherm have been formulated but relative size factor,  $x$  (the ratio of the size of adsorbate and solvent) has been ignored. Precise evaluation of the standard free energy, ( $\Delta G^\circ$ ) of solvent-substitution adsorption process at the charged interface, from the experimental surface coverage ( $\theta$ ) data cannot be made unless the size factor  $x$ , in  $\theta$  is properly formulated. Levine *et al.*<sup>7</sup> first recognised the importance of size factor for electrochemical adsorption. Evaluation of thermodynamical parameters for the inhibition process is of great importance and hence an attempt is made in this communication to evaluate free energy of adsorption of BTA on copper single crystal planes during acid corrosion.

Solutions were prepared from freshly distilled and pre-electrolysed AR sulphuric acid and recrystallised BTA (Merck) using triple distilled water. 99.999% copper (110), (100) and (111) planes with dislocation density of the order  $10^6/\text{cm}^2$  were mechanically polished on 4/0 emery paper using ethyl alcohol as lubricant and then electro-polished in 1:1 orthophosphoric acid at a cell potential of 1.2 V for 30 minutes. The dissolution

was carried out in aerated 0.1 N sulphuric acid with desired amount of BTA. The detailed experimental procedure has been given in a previous communication<sup>8</sup>.

Copper (110), (100) and (111) planes were dissolved in aerated unstirred 0.1 N sulphuric acid containing various concentrations ( $10^{-3}$  to  $7.5 \times 10^{-3}$  M) of BTA at different temperatures and dissolution rates ( $\text{mg}/\text{cm}^2/\text{hr}$ ) were evaluated. The surface coverages ( $\theta$ ) of BTA on different single crystal planes were evaluated from the dissolution rates data using the equation<sup>9</sup>

$$\theta = 1 - P/P_0$$

where  $P$  and  $P_0$  were the dissolution rates with and without BTA respectively. Surface coverages of BTA on copper single crystal planes at 30°C are given in Table I. The apparent free energy

TABLE I  
Surface coverages of BTA on copper single crystal planes in 0.1 N sulphuric acid at 30°C

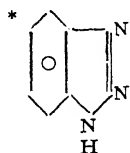
Concentration of BTA	$\theta$		
	(110)	(100)	(111)
$10^{-3}$ M	0.4706	0.4329	0.1236
$1 \times 10^{-3}$ M	0.5294	0.5561	0.1533
$2 \times 10^{-3}$ M	0.5883	0.6548	0.2275
$3 \times 10^{-3}$ M	0.6470	0.7657	0.2869
$4 \times 10^{-3}$ M	0.7059	0.8644	0.3262
$5 \times 10^{-3}$ M	0.7451	0.9260	0.4207
$7.5 \times 10^{-3}$ M	..	..	0.5841

of adsorption ( $\Delta G_a^\circ$ ) of BTA on copper single crystal planes at different concentrations of BTA were evaluated using the equation<sup>10</sup>.

$$\Delta G_a^\circ = -2.303 RT \log \left[ \left( \frac{55.4\theta}{C_{\text{org}}(1-\theta)^x} \right) \left( \frac{\theta + x(1-\theta)^{x-1}}{x^x} \right) \right]$$

where  $C_{\text{org}}$ , the concentration of organic compound in the bulk of the solution and  $x$ , the size factor. From the values of molecular weights, densities and molecular radii of water and BTA,  $x$  was calculated and it found to be 5. Figure 1 shows the variation of apparent free energy of adsorption of BTA with surface coverage ( $\theta$ ) on copper (110), (100) and (111) planes. The apparent negative free energy of adsorption was different on different single crystal planes and it was more negative on the (100) plane than on other planes.

Electrochemical adsorption always involves solvent displacement<sup>11</sup> at the interface. Thermodynamics of the exchange of adsorbate between the bulk and the interface therefore depends on the relative size of adsorbate and solvent<sup>12</sup>.



The competitive (substitution) adsorption of BTA molecules from solution is similar to the general quasi-chemical substitution process<sup>10</sup>.

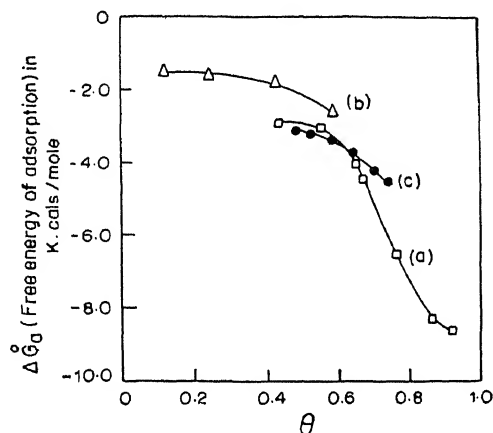
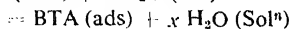
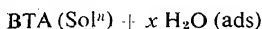


FIG. 1. Variation of free energy of adsorption of BTA with surface coverage on (a) (100) plane; (b) (111) plane; (c) (110) plane.

Corrosion inhibition follows the specific adsorption of inhibitor molecules at the reaction sites (defects) on the surface of the crystal plane. The extent of corrosion inhibition is proportional to the extent of adsorption of BTA on the surface of copper single crystal plane, which in turn depends on the number of surface defects. The number of surface defects depends on the crystallographic orientation of the substrate<sup>13</sup>. Hence we could expect different rates of adsorption and inhibition of BTA on single crystal planes. Since the free energy of adsorption is more negative on the (100) plane, it is expected that there is more spontaneous adsorption and inhibition by BTA on the (100) plane than on other planes. This is in agreement with the experimentally observed higher order of inhibitor efficiency of BTA on the (100) plane than on other planes.

The present work carried out was inspired by late Professor T. H. V. Setty. The author wishes to express his grateful thanks to Dr. M. Shadaksharaswamy, Professor and Head of the Department of Chemistry, Central College, Bangalore, for his kind encouragement.

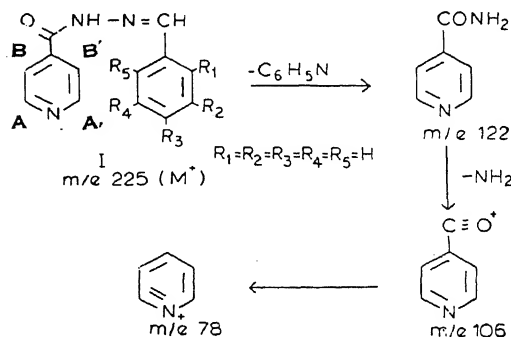
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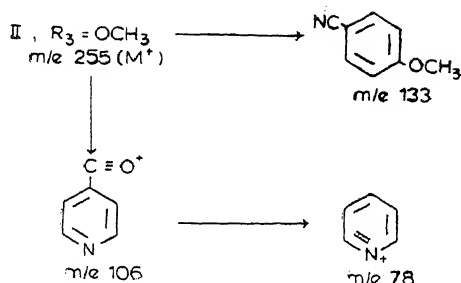
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#### MASS-SPECTRAL FRAGMENTATION PATTERN OF SOME SCHIFF BASES FROM ISONIAZIDE AND OF BENZALAZINES

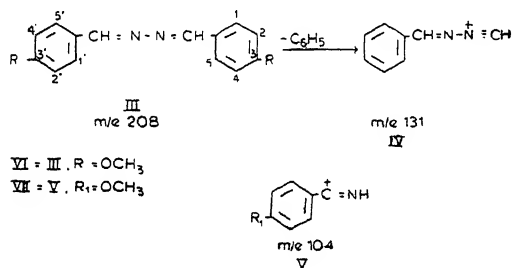
In a previous communication we have reported the preparation and anti-tuberculous activity of a number of new Schiff bases obtained by the reaction of isoniazide with different aromatic aldehydes<sup>1</sup>. We have also reported that these Schiff bases on treatment with dilute alkali give rise to benzalazines<sup>2</sup>. For the identification of the latter we happened to take their mass spectra and also compare them with those of the Schiff bases. The fragmentation patterns in both cases are of interest since the Schiff bases also contain an amide grouping while the benzalazines have two  $-\text{N}=\text{CH}-$  groups. No information on the mass spectral data of such compounds is available so far and is reported in the present paper. In the case of the Schiff base I, in addition to the molecular ion peak at  $m/e$  225, three other prominent peaks are obtained the genesis of which may be rationalised according to the fragmentation pattern indicated below<sup>3</sup>:



Similarly, in the mass spectrum of II the fragmentation pattern can be explained as,



The benzalazine III from I showed peaks in the mass spectrum as indicated. The loss of a phenyl radical leads to the ionised fragment IV. This is known in the case of anils of benzaldehyde where a peak corresponding to loss of an aryl group is obtained<sup>8</sup>.



IV on loss of HCN leads to the ion V which again on loss of HCN leads to the phenyl cation corresponding to the peak at  $m/e$  77.

In a similar manner, the benzalazine VI gives a molecular ion peak at  $m/e$  268. The loss of the *p*-methoxy phenyl radical leads to the ion corresponding to the peak at  $m/e$  161 which loses HCN to give the ion VII which further loses HCN to afford the phenyl cation at  $m/e$  77.

As a point of interest we also give the NMR spectral data of compounds I and III. The latter can exist in the alternate tautomeric form. I: NMR (DMSO) ppm, 7.49 (3 H, m), ( $\text{C}_2\text{H}$ -,  $\text{C}_3\text{H}$ -, and  $\text{C}_4\text{H}$ -); 7.75 (2 H, d,  $J = 7.5$  Hz), ( $\text{C}_1\text{H}$ - and  $\text{C}_5\text{H}$ -); 7.84 (2 H, d,  $J = 7$  Hz), ( $\text{C}_6\text{H}$ - and  $\text{C}_4\text{H}$ ); 8.53 (1 H, s), ( $-\text{N}=\text{CH}$ ); 8.83 (2 H, dd,  $J = 7$  Hz and  $J = 1$  Hz) ( $\text{C}_A\text{H}$ - and  $\text{C}_X\text{H}$ -); 12.13 (1 H, s), ( $=\text{NH}$ ).

III: NMR (DMSO) ppm 7.58 (6 H, m), ( $\text{C}_2\text{H}$ -,  $\text{C}_3\text{H}$ ,  $\text{C}_4\text{H}$ -,  $\text{C}_2$ , H,  $\text{C}_3$ , H and  $\text{C}_4$ , H); 7.94 (4 H, m), ( $\text{C}_1\text{H}$ -,  $\text{C}_5\text{H}$ -,  $\text{C}_1$ , H and  $\text{C}_5$ , H); 8.66 (2 H, s), ( $-\text{N}=\text{CH}$ ).

The above spectrum indicates that the benzalazine III does not exist in the ketimine form.

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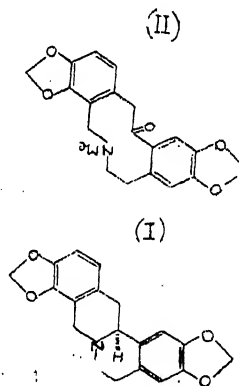
### SEED ALKALOIDS OF *FUMARIA INDICA*

IN continuation of our studies<sup>1-8</sup> on the chemical constituents of *Fumaria indica* (Haussk.) Pugsley<sup>4</sup>, we now report our findings on the alkaloids of the seeds of this plant. It is only after our first communication<sup>1</sup> on the isolation of protopine along with six other tertiary bases from the whole plant of *F. indica* that Satish and Bhakuni<sup>5</sup> reported the presence of protopine, its methohydroxide, non-acosanol and sitosterol from the stems and leaves of this plant. No work on the seeds of this plant has yet appeared.

Air-dried seeds (0.75 kg) of *F. indica* were powdered and extracted in a soxhlet extractor successively with petroleum ether and rectified spirit. Petrol extract was concentrated to a thick syrup and churned mechanically with 7% aqueous citric acid. The aqueous acid was filtered, washed with light petrol, rendered basic with ammonium hydroxide and extracted exhaustively with ether. The ether extract containing the alkaloid mixture was dried over anhydrous sodium sulphate, evaporated to dryness and subjected to chromatographic resolution over Brockmann neutral alumina, monitoring at every stage for homogeneity of the eluates over silica gel G chromatoplates. Fractions eluted with petrol-benzene (1:1) mixture furnished a solid ( $R_f$ , 0.70 in methanol) which crystallised from chloroform-methanol as needles. m.p. 200–202° (yield, 0.05%). The alkaloid,  $\text{C}_{19}\text{H}_{17}\text{NO}_4$ , ( $M^+$ , 323), absorbed light in the UV region,  $\lambda_{\text{max}}$  235 sh, 288 nm (log  $\epsilon$ , 3.27, 3.22), and showed in its 60 MHz PMR spectrum two methylenedioxy groups (2 H singlets at 6.03 and 6.06 ppm) and four aromatic protons (1 H at 6.71, 2 H at 6.78 and 1 H at 6.88 ppm). The mass spectrum of the alkaloid, besides the molecular ion peak at  $m/e$  323 (44%), showed a significant M-1 peak at  $m/e$  322 (23%). The molecule underwent straightforward fragmentation into two clean halves with generation of peaks at  $m/e$  174 (14%)

and 148 (100%), presumably by retro-Diels-Alder cleavage<sup>6</sup> with concomitant loss of one hydrogen from the dihydroisoquinoline moiety needed for stabilisation of the ion. The genesis of the peak at  $m/e$  149 (15%), discernible in the spectrum, is considered to be due to capture of one hydrogen by the base peak. The data strongly suggest the identity of the alkaloid with tetrahydrocoptisine and, in fact, it was indistinguishable from *dl*-tetrahydrocoptisine<sup>3</sup>, m.p. 221–222°; isolated from the whole plant, by TLC and spectral studies. Based on this observation and the fact that the alkaloid showed a negative rotation,  $[\alpha]_D - 339^\circ$  (c. 0.308 in  $\text{CHCl}_3$ ), it was identified as 1-tetrahydrocoptisine (I).

The second alkaloid eluted out of the column with petrol-benzene (3 : 7) and crystallised from chloroform-methanol mixture as stout prisms, m.p. 206–208°. It was identified as protopine (II) by direct comparison (m.p., mixed m.p. and superimposable IR).



The rectified spirit extract of the seed was processed identically as the petrol extract and the only alkaloid that could be obtained from this fraction was protopine. The total yield of protopine from the seeds was found to be around 0.15%.

The work shows that while the protopine content of the seeds is about double that of the whole plant, the yield of tetrahydrocoptisine is 50 times more in seeds than in the whole plant. More importantly, the latter is present as an optically active form in seeds rather than as a racemic mixture.

Sincere thanks are due to Professor G. B. Singh of the Department of Chemistry for providing helps in having UV, IR and PMR spectra and to Dr. D. P. Chakravarty, Bose Research Institute, Calcutta, for measurement of specific rotation. Mass spectrum was recorded in the National Chemical Laboratory, Poona. Financial

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#### CHEMICAL CONSTITUENTS OF *ACTINIOPTERIS RADIATA* (SW.) LINK.

*Actiniopteris radiata* (Actiniopteridaceae) is a common desert fern available in Rajasthan. Locally it is known as Morepankhi. Medicinally it is anthelmintic and styptic and is a common cattle-feed. Previous work<sup>1</sup> has shown the presence of rutin in this plant. In the present communication we report the presence of hentriacontane, hentriacontol,  $\beta$ -sitosterol palmitate,  $\beta$ -sitosterol,  $\beta$ -sitosterol-D-(+)-glucoside, an unidentified glucoside, glucose and fructose.

Dried stems and leaves of the plant (500 g) were extracted with petroleum ether (40–60° C) and ethanol respectively. The petroleum ether extract was concentrated under reduced pressure to a green solid mass (10 g). It was put over an alumina column and eluted successively with pet. ether (40–60° C), pet. ether : benzene (4 : 1), benzene and benzene : chloroform (1 : 1).

The first few fractions from pet. ether on evaporation gave a waxy compound (20 mg). It was crystallised from acetone, mp 66° C. IR (KBr) shows bands at 2920  $\text{cm}^{-1}$ , 2850  $\text{cm}^{-1}$ , 1465  $\text{cm}^{-1}$ , 1380  $\text{cm}^{-1}$ , 725  $\text{cm}^{-1}$  and 714  $\text{cm}^{-1}$ ; indicating it to be a long chain *n*-alkane hydrocarbon<sup>2</sup>. It was identified as hentriacontane (mp, mmp. and superimposable ir).

The fractions after elution with pet. ether : benzene (4 : 1) gave a compound (100 mg), mp 86° C which was recrystallised from acetone. It gives red colour in Liebermann-Burchard test and yellow colour with tetranitromethane, indicating the steroidal nature of the compound. The IR spectrum in KBr gives, beside other bands, a peak at 1740  $\text{cm}^{-1}$ , indicating the presence of an ester

grouping. The compound on hydrolysis with 2% alcoholic solution of KOH furnished  $\beta$ -sitosterol and palmitic acid. The identity of the compound was confirmed as  $\beta$ -sitosterol palmitate by comparing it with its authentic sample (mp, mmp, cotlc and superimposable ir), which was prepared by refluxing  $\beta$ -sitosterol and palmitic acid in benzene, in presence of *p*-toluenesulphonic acid as a catalyst<sup>3,4</sup>.

The elutes from pure benzene on evaporation gave another compound (100 mg), crystallised from acetone mp 84° C. The band at 3400 cm<sup>-1</sup> in ir (KBr) shows the presence of alcoholic group in the molecule. It was identified as hentriacontol by comparison (mp, mmp, cotlc and superimposable ir).

The fractions from benzene : chloroform (1 : 1) furnished a compound (50 mg), crystallised from chloroform, mp 136° C, acetate mp 128° C, gives positive Liebermann-Burchard and tetranitromethane test. It was identified as  $\beta$ -sitosterol (mp, mmp, cotlc,  $[\alpha]_D$  and superimposable ir).

The alcoholic extract of the plant was concentrated to one-tenth of its original volume and was kept at 0° C for few days. A yellow crystalline substance was accumulated at the bottom. This on repeated crystallisation from methanol gave a pale yellow crystalline substance mp 190° C. It failed to give the tests for steroids and flavonoids. However positive response to Molisch's test and blood red colouration with conc. H<sub>2</sub>SO<sub>4</sub> indicates the glycosidic nature of the compound. The IR spectrum of the compound further confirms it<sup>5</sup> (bands 3650 cm<sup>-1</sup>, 3400 cm<sup>-1</sup>, 1275 cm<sup>-1</sup>, 1225 cm<sup>-1</sup>, 1050 cm<sup>-1</sup>, 975 cm<sup>-1</sup>, 900 cm<sup>-1</sup>, 850 cm<sup>-1</sup>). The NMR of the compounds in DMSO gives a signal at 5.1  $\delta$ , 1 H(d) and a multiproton multiplet between 3-4  $\delta$  showing the presence of glucose moiety<sup>6</sup>. The signal at 3.5  $\delta$ , 3 H(S) corresponds to one—OMe grouping. On hydrolysis with 2N HCl compound gives glucose, along with an unidentified black residue. Further work is in progress on its structure elucidation.

The remaining part of the alcoholic extract was concentrated to dryness and put over a silica gel column. Elution with chloroform : methanol (10 : 1) resulted in a compound (20 mg), which was crystallised from ethyl acetate mp 290° C, acetate mp 160° C. Positive response to Liebermann-Burchard and Molisch's tests indicates that it is a steroidal glycoside. On hydrolysis with 4N HCl it gives glucose and  $\beta$ -sitosterol. This was confirmed to be  $\beta$ -sitosterol-D(+)-glucoside by mp, mmp, cotlc and superimposable ir with the authentic sample and acetate.

The presence of glucose and fructose in the alcoholic extract was confirmed by paper chromatography methods.

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#### SOME BIOLOGICAL OBSERVATIONS ON THE MITE, *ACARAPIS WOODI* RENNIE, INFESTING HONEYBEES, *APIS CERANA INDICA* F.

*Acarapis woodi* is an internal parasite of honeybees and infests both *Apis mellifera* L. and *Apis cerana indica* F. It is found all over the world excepting North America, Scandinavia and Australia<sup>3</sup>. From India, it was first reported in 1957 from the Simla Hills<sup>1,2</sup>. Acute infestation causes 'acarine' disease resulting in rapid dwindling and mortality of bee colonies<sup>4,6</sup>. Considerable studies have been made on its biology, behaviour, and control in the European countries, which are reviewed by Bailey<sup>1,2</sup>. With a view to understanding the problem under diverse conditions in India, efforts were made to study biological behaviour of the mite infesting *A.c. indica* under mid-hill conditions at Solan (latitude 31° N, longitude 77.2° E, altitude 1540 m).

1. *Susceptibility of different age groups of honeybees to mite infestation.*—Two thousand newly emerged bees were obtained by placing sealed brood in an incubator at 32° C. They were divided into four groups of 500 each and marked on thoraces with distinguishing colours. One group was released immediately and the remaining were released successively in a colony with 40-50% infestation so that age of bees when released was 0, 7, 14 and 21 days. Seven days after release, 250 bees were recovered for examination.

Per cent infestation is recorded in Table I. Bees upto 7 days age were most susceptible with 41.9% infested bees. The incidence was 11.6% in bees aged 7-14 days. Bees in the age group of 14-21 days were least susceptible while those older than 21 days did not contract infestation.

TABLE I

*Susceptibility of different age-groups of bees to A. woodi infestation*

Age-group of bees (days)	Number of bees examined	Number of bees found infested	Per cent infestation
0-7	217	91	41.9
7-14	189	22	11.6
14-21	231	3	1.3
21-28	209	..	0.0

II. *Progress of infestation in newly emerged bees.*—One thousand just-born bees were released in an infested colony after marking. Infestation progress was recorded for 7 days, every 12 hours for the first 3 observations and thence every 24 hours, by recovering 50 marked bees and examining the mites in their trachae.

Data (Table II) show that bees remained free of mites upto 24 hours age while 8% caught the infestation when under 36 hours old. Progress of further infestation was 22, 38, 36, 42, 38 and 40% for 2, 3, 4, 5, 6 and 7 days old bees respectively.

TABLE II

*Progress of infestation in newly emerged bees*

Age of bees	Number of bees examined	Number of bees found infested	Per cent infestation
12 hours	50	..	0.0
24 "	50	..	0.0
36 "	50	4	8.0
2 days	50	11	22.0
3 "	50	19	38.0
4 "	50	18	36.0
5 "	50	21	42.0
6 "	50	19	38.0
7 "	50	20	40.0

It shows that susceptibility of bees to mite attack increased from the 2nd day after emergence and reached the peak by the 3rd through 4th day where after it declined while the percentage of already infested bees remained fairly constant. It is interesting to note that all the bees were not infested. Lack of infestation in bees under 24 hours old has also been reported earlier<sup>9</sup>, and may be due to their comparative inactivity during the first day of their life, which they spend mostly in cleaning themselves and resting on brood combs<sup>8,10,11</sup>. Owing to inactivity they probably did not come into contact with older bees having mature mites clinging to their body hair-tips<sup>2</sup>. Also, the spiracular openings of bees under 24 hours old may not be large enough to permit entry of mites.

III. *Surviving ability of the mite in dead honeybees.*—The infested bees crawl about being

unable to fly due to disjointed wings. Such crawlers were collected on different dates in batches of 120. They were killed by severing their heads. Twenty bees were examined every two hours till they were dead for 96 hours.

TABLE III

*Survival of A. woodi in dead honeybees*

Batch number of diseased bees	Date/time of killing the diseased bees	Examination of diseased bees for living mites	Time elapsed since death of the examined bees (hours)	Number of bees examined	Number of bees having living mites
I	3-8-73/0700	3-8-73	0900	2	20
			1100	4	20
			1300	6	20
			1500	8	20
			1700	10	18
			1900	12	13
II	3-8-73/1900	4-8-73	0900	14	26
			1100	16	20
			1300	18	20
			1500	20	20
			1700	22	20
			1900	24	20
III	3-8-73/0700	5-8-73	0900	26	20
			1130	28	20
			1300	30	20
			1500	32	20
			1700	34	20
			1900	36	20
IV	4-8-73/1900	6-8-73	0900	38	20
			1100	40	20
			1300	42	20
			1500	44	20
			1700	46	20
			1900	48	20

Most of the mites were found dead within 24 hours of the death of their hosts (Table III). No mite was observed surviving on honeybees dead for over 42 hours. Earlier reports<sup>5,7</sup> state that the mite leaves the trachea of the dead bee and searches for a new host. In our opinion, infestation through this mode is not of much consequence as the dead bees fall away from the cluster or the dying bees crawl out of hives prior to death.

These investigations, therefore, revealed that : (i) 2-7 days old *A.c. indica* bees are most susceptible to *A. woodi* infestation; (ii) incidence is highest in 3-4 days old bees; and (iii) dead honeybees may not be a significant source of spreading infestation.

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#### EFFECT OF ROOT KNOT EXTRACT OF GINGER ON *FUSARIUM OXYSPORUM* f. *ZINGIBERI* TRUJILLO CAUSING YELLOW DISEASE

YELLOW DISEASE of ginger (*Zingiber officinale* Rosc.) is fairly common in Madhya Pradesh. Root knot nematodes have also been reported to be associated with the diseased samples by Haware and Joshi (1972). It has been reported that the nematodes increase the severity of Fusarium wilt in several crops (Powell, 1971). Hence it was deemed essential to see the influence of nematode infection of ginger roots on the vigour of the pathogen, *F. oxysporum* f. *zingiberi*.

The larvae of *Meloidogyne incognita* Chitwood were collected from surface sterilized galled roots of ginger as per procedure adopted by Thomson *et al.* (1959). After collection and surface sterilization, larvae were added in the sterilized soil in earthen pots. In addition, 25 g of surface sterilized galled roots of ginger were added in each pot. Larvae and galled roots were surface sterilized with 3% acid free hydrogen peroxide for 30 min as described by Byaras (1914). In control, nematodes were not added. Surface-sterilized rhizome pieces were sown in each pot, and examined after 30 days for gall formation. Severe gall formation was observed on roots from infested soil while they were absent on roots from non-infested soil. Roots from both the treatments were collected separately, washed in running water and surface sterilized. Tender galled roots were selected, crushed and the juice was extracted for preparing the galled root extract medium. Similarly non-galled roots extract medium was prepared from the healthy ginger roots. These

media were used for studying the growth of *F. oxysporum* f. *zingiberi*. The inoculated plates were incubated at 25° C. The radial growth of colonies was recorded after 3 and 5 days of incubation. The thickness of hyphae was also measured on 5th day. The experiment was repeated twice and the mean data are presented in Table I.

TABLE I

*Effect of healthy and nematode infected root extract medium on growth and hyphal thickness of F. oxysporum f. zingiberi*

Medium	Average radial growth of colony in mm after		Hyphal thickness in $\mu$
	3 days of incubation	5 days of incubation	
Galled root extract	31.4	81.0	4.31
Healthy root extract	15.4	57.5	3.75

It is evident from the data that linear growth and hyphal thickness of the pathogen was greater in galled root extract than in the healthy roots extract medium. It indicates the presence of some growth promoting substance in the galled roots which is produced by the interaction effect of host and nematodes. These results are in accord with those reported by Melendz and Powell (1965) in case of tobacco.

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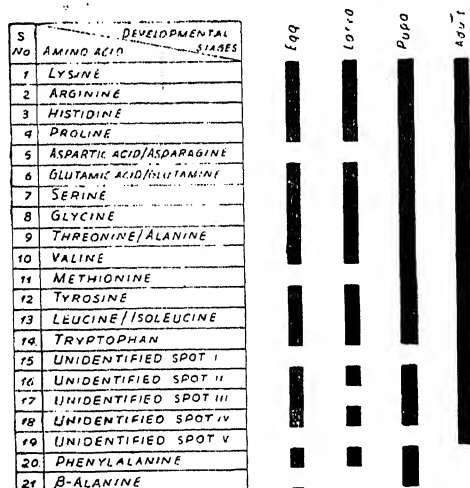
#### FREE AMINO ACIDS IN THE DEVELOPMENTAL STAGES OF *ORYZAEPHILUS SURINAMENSIS* (L.)\*

A CHARACTERISTIC feature of the insect haemolymph is the presence of high concentration and wide variety of amino acids. The pattern of free amino acids is not the same in all the insects but varies with different orders<sup>1</sup>, developmental stages<sup>2</sup>, and

the food intake<sup>3,4</sup>. It is even under genetic control in some cases. The present investigation was undertaken to study the qualitative variation in the free amino acids in different developmental stages of saw toothed grain beetle (*Oryzaephilus surinamensis* L.) which would be helpful in elucidating the basic processes underlying the insect development.

The analysis was done on 48 hour old eggs, mature larvae, pupae and adult. 200 mg (dry weight) of each stage were taken. Proteins and fats were removed<sup>5</sup> and the aqueous fraction was stored for subsequent analysis of free amino acids. Air dried silica gel-G plates of 250  $\mu$  thickness were subjected to two-dimensional chromatography for identifying individual amino acids. The solvent used in the first run was a mixture of *n*-butanol : acetic acid : H<sub>2</sub>O (80 : 20 : 20, v/v) and in the second run, the mixture consisted of phenol : water (75 : 25, W/v). To prevent the oxidation, 20 mg of NaCN were added to the second solvent. Ethanol ninhydrin solution (0.2%) was used to detect the spots. These spots were identified by comparing their  $R_f$  values with those of the authentic samples. Since the  $R_f$  value of pure substances is not constant (Brenner *et al.* 1962)<sup>6</sup> and is affected by length of run, changes in temperature and other environmental factors; the identity of amino acids was also confirmed by co-chromatography.

As is evident from Fig. 1, in *O. surinamensis*, in spite of all the morphogenetic processes going



Qualitative free amino acid in *Oryzaephilus surinamensis*

FIG. 1

on during insect development, there is a constant occurrence of most of the nutritionally essential

and non-essential free amino acids. Only a few variations or exceptions can be noted.

In the adult stage, exception is phenylalanine, its absence may be expected on the basis that dietary phenylalanine gets converted (hydroxylation) readily into tyrosine in the insect body which leads to the identification of tyrosine and not of phenylalanine<sup>7</sup>. Absence of phenylalanine in adults is also reported earlier<sup>8</sup>.

In eggs all amino acids, except methionine and aspartic acid found in the adults, are present. This is expected since the blood proteins have been reported to be directly deposited in the yolk of the egg<sup>9</sup>. Since methionine is an indispensable amino acid for the production of eggs<sup>10</sup>, it seems probable that the stock of the methionine must have been completely reduced, or even exhausted. The egg, being a closed system, cannot procure the amino acid from any external source. The absence of methionine has also been reported from the eggs of *Drosophila melanogaster*<sup>11</sup>.

TABLE I

Average  $R_f$  values of standard amino acids

Amino acid		1st Run	2nd Run
		Butanol : Acetic acid : H <sub>2</sub> O (80 : 20 : 20 v/v)	Phenol : H <sub>2</sub> O (75 : 25 W/v)
1. Lysine	..	0.03	0.09
2. Arginine	..	0.05	0.20
3. Histidine	..	0.06	0.24
4. Proline	..	0.14	0.49
5. Aspartic acid/Asparagine		0.17	0.05
6. Glutamic acid/glutamine		0.24	0.07
7. Serine	..	0.18	0.19
8. Glycine	..	0.18	0.23
9. Threonine/alanine	..	0.21	0.29
10. Valine	..	0.32	0.39
11. Methionine	..	0.35	0.47
12. Tyrosine	..	0.41	0.44
13. Leucine/isoleucine	..	0.42	0.49
14. Tryptophan	..	0.46	0.51
15. Phenylalanine	..	0.50	0.52
16. $\beta$ -alanine	..	0.26	0.29

Average  $R_f$  values of unidentified spots

17. Unidentified spot I	..	0.14	0.26
18. " II	..	0.15	0.31
19. " III	..	0.20	0.34
20. " IV	..	0.10	0.37
21. " V	..	0.29	0.37

The absence of aspartic acid in the eggs and mature larvae may probably be due to its conversion to L-alanine by decarboxylation as has been reported in *Bombyx mori*<sup>12</sup> or by transamination to glutamic acid. The metabolism through these conversions can lead to the identification of alanine and glutamic acid and not aspartic acid. Methionine,

essential for pupation and the emergence of adults, is absent in larvae. Since L-methionine can be replaced at least by L-serine or glycine<sup>13</sup>, the absence of methionine is expected which shows the presence of glycine and serine but not methionine.

One of the characteristic features is the presence of  $\beta$ -alanine in pupa, which is a part of pantothenic acid in essential vitamin for *O. surinamensis*<sup>14</sup>.

Other unidentified ninhydrin positive spots recorded in different developmental stages may be peptides or amino acid derivatives resulting from proteolysis of the body tissue.

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#### AN APPROACH TO INCREASE THE EFFICIENCY OF INDUCTION OF MUTATIONS IN RICE (*ORYZA SATIVA* L.)

To develop a technique of mutagenic treatment which would reduce the degree of damage to the treated material and maximise the production of mutations (macro and micro), comprehensive experiments were conducted with a few *indica* rice varieties by the use of various combinations of single and recurrent radiation and chemical mutagens which have been reported partly earlier<sup>4</sup>. An approach for recurrent radiation treatment which appeared to be most useful after repeated tests is described.

Dry (14 p.c. moisture) dormant rice seeds, irradiated with a low radiation dose (5 kR), were

grown and seed samples, collected from primary tillers of a number of normal-looking  $M_1$  plants, were pooled together and reirradiated with respective doses (5, 15, 30 kR) of X- and  $\gamma$ -rays to obtain  $M_{1/2}$  plants. Appropriate controls were also tested. From the observations on various growth metrics in  $M_1$  and  $M_{1/2}$  generation and frequency of macro (chlorophyll) and micro (variability for quantitative traits) mutations in  $M_3$  and  $M_{2/3}$  generation, it was evident that the background irradiation with low dose (5 kR) imparted a marked radio-resistance, as expressed by increase in germination, survival and spike fertility (Table I). Moreover,

TABLE I  
Effects on growth and chlorophyll mutation frequency in  $IR8$

Treat- ment*	Germination†	Survival	Seedling height	Dry weight‡	Seed fertility	Frequency of chlorophyll mutation§
0	100.0	100.0	100.0	100.0	100.0	0.0
5	92.4	85.4	98.5	90.2	81.4	3.5
15	79.2	75.7	72.6	72.7	56.8	16.2
5+15	90.4	86.8	95.5	79.9	77.0	14.4
30	71.6	63.1	57.8	68.3	38.8	18.5
5+30	87.8	80.4	74.0	76.8	63.4	19.5
30+30	60.4	66.3	60.0	67.5	44.6	19.1

\* Dose in kR; † 200/400 seeds sown; ‡ 35 days old seedlings; § Per 1000  $M_2$  seedlings.

the scope for isolating higher number of mutants due to increase in survival and marked increase in genetic variance without affecting much the viability of the population, as expressed by the mean value of a trait, was evident (Table II).

Following the suggestion of Caldecott and North<sup>1</sup> for recurrent irradiation as a tool of distinct value to the plant breeder, several scientists have used this with different crops<sup>2-6</sup> with limited success. The failure to achieve substantial gain with recurrent irradiation in mutation breeding might be in many instances due to deleterious effects caused by high-dose-induced increased radio-sensitivity, as also evident from the present data. Although from the literature<sup>5,7</sup> it is evident that acquired radio-resistance has been observed by several scientists in various organisms, attempts have been rarely made to exploit this tool in breeding of crop plants, specially in rice. Several promising mutant strains with regard to yield, grain quality, earliness, moderate resistance to diseases have been isolated following this approach<sup>3,8</sup>.

TABLE II

Effects on micro-mutation in  $M_3$  and  $M_{2/3}$ -grain yield per plant\*

Dose kR of X-rays	Mean (g)	$\sigma g^2$	G.A.
0	10.2 <sup>(1)</sup> 7.1 <sup>(2)</sup>	.. ..	.. ..
5	10.1 7.0	0.96 2.42	0.67 1.53
15	8.7 6.6	3.47 4.47	1.99 2.70
30	8.4 5.9	4.62 5.02	2.19 3.01
5+5	10.3 6.8	1.10 2.33	0.79 1.70
15+15	8.8 6.0	4.87 5.40	2.66 3.16
30+30	7.9 5.7	3.97 3.56	2.01 2.16
5+30	9.5 6.6	6.69 8.20	2.99 4.23

\* In total 527 and 456 lines were studied in T (N)1 and Dhairal respectively.

(1) T (N) 1 (2) Dhairal;  $\sigma g^2$ =Genetic variance; G.A.=Genetic advance.

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# RECORD OF THE BATHYPELAGIC FISH. EPINNULA ORIENTALIS GILCHRIST AND VON BONDE FROM THE BAY OF BENGAL

FISHES belonging to the genus *Epinnula* Poey are the smallest among the gempylids and are considered rather rare as they are seldom caught. During a recent survey of the Tamil Nadu coast, the capture of numerous examples of *Epinnula orientalis*

Gilchrist and von Bonde, belonging to the family Gempylidae, from the continental slope off Madras in the Bay of Bengal, by the otter trawl operated at 250 metres, on board the *Red Snapper* of the Central Institute of Fisheries Operatives, Madras Unit, is, therefore, worthy of note. The present communication records *Epinnula orientalis* for the first time from the Bay of Bengal hitherto previously being reported from off Natal and Delagoa Bay (South Africa), and off the south-west coast of India in the Indian Ocean; from the Pacific off Japan and the Philippines; and from the Atlantic in the Gulf of Mexico (Herre<sup>1</sup>, Grey<sup>2</sup>, Narayana-Rao<sup>3</sup>).

*Epinnula orientalis* Gilchrist and von Bonde

*Epinnula orientalis* Gilchrist and von Bonde<sup>4</sup>, 1924, 15, pl. 4 (1); Smith<sup>5</sup>, 1953, 311, fig. 865; Narayana-Rao<sup>3</sup>, 1965, 217.

**Material**—116 exs, 66–112 mm in standard length, off Madras, 250 metres, 31, January 1974, coll. P. K. Talwar; Zoological Survey of India, regd. no. F. 7108/2.

**Description**—D XVI. I. 18–20; A III. 18–20; P 14; Gill rakers on first arch, one in angle followed by 6 fine rakers.

Depth of body 22.4–26.0, head length 31.1–35.6, tip of snout to origin of dorsal fin 30.6–31.6, tip of snout to origin of anal fin 70.9–73.4, origin of anal fin to caudal base 27.2–29.2, eye diameter 5.4–7.0, snout length 10.9–12.1, interorbital width 6.1–6.6, length of pectoral fin 12.6–16.3; all in percentage of standard length. Eye diameter 17.4–22.2, snout length 30.6–36.6, interorbital width 18.0–20.8, and pectoral fin length 38.4–45.8; all in percentage of head length.

Body fusiform and compressed. Cleft of mouth oblique, large, lower jaw projecting, maxillary extending to below vertical from anterior third to half the eye diameter. Two lateral lines, originating together above the upper angle of the gill opening; the upper branch running parallel to dorsal profile of the body, extending slightly beyond the end of second dorsal fin, the lower one running along the ventral edge of the body, extends to the base of the middle rays of the caudal. **Teeth**: jaws anteriorly set with fang-like teeth, three on the upper and two on the lower; other teeth are small, sharp and widely spaced in a single row; teeth of the lower jaw somewhat larger than those of the upper; vomer and palatines dentate, two small teeth on vomer and a single row of 6–8 minute, fine teeth on each palatine. **Fins**: first dorsal fin depressible in a groove, base longer than second dorsal fin base; origin of second dorsal fin slightly behind vertical from anal fin origin; pelvic fin origin below vertical from midlength of

pectoral fin, origin closer to vent than to tip of snout. Scales : minute, cycloid, deciduous, so that the specimens are practically naked.

Colour : in alcohol, uniformly dark brown ; spinous dorsal fin dusky, other fins pale ; opercular lining dusky, inside of mouth somewhat so.

Remarks.—Grey<sup>2</sup> established two subspecies of *Epinula orientalis* Gilchrist and von Bonde based on material from the Pacific and Atlantic, and distinguished these subspecies from the typical form known from the western Indian Ocean, in having the ventral fin below the vertical from the middle of the pectoral fin (*versus* behind the tip of the pectoral fin). Apparently Grey<sup>2</sup> used only literature description for *E. orientalis orientalis* since Smith's<sup>5</sup> figure of a topotype clearly shows the ventral fin below the vertical from the midlength of the pectoral fin. The original description and figure of *E. orientalis* seems to be faulty in this character. In the specimens from the Bay of Bengal and also in the 26 specimens collected from off the south-west coast of India during 1971 by one of us (PKT) (ZSI regd. No. F. 6295/2), the ventral fin is also inserted below the vertical from the midlength of the pectoral fin. Comparison of the Indian material with the type descriptions of *E. orientalis pacifica* and *E. orientalis americana* indicates that they are conspecific since the Indian Ocean subspecies embraces the diagnostic characters of the Pacific and Atlantic Ocean subspecies ; the differences seem to be based on inadequate material and local variations.

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## JUTE SEED STORAGE AND OXYGEN REQUIREMENTS

It is a common practice to store jute seeds loosely in containers and some open space is left deliberately on the supposition that jute seeds respire, though very feebly, during storage and their viability is affected if totally cut off from oxygen. In a seminar, the question was posed that if fungal spores remain viable for years under perfectly anaerobic conditions (as under paraffin oil) why jute seeds should need oxygen for respiration. It was said that fungal spores and jute seeds are morphologically quite different and so they are also different in their physiological activities.

In the light of the above discussions, it was thought necessary to study the point very critically. Two varieties of jute seeds were selected for testing ; they are *Corchorus olitorius* JRO-632 and *C. capsularis* JRC-321. The moisture contents of the two varieties were between 9–10%, which is known to be a range at which the viability of jute seeds during storage is not affected<sup>1</sup>.

Three sets of experiments were carried out. In set No. 1 the seeds were very tightly packed in glass bottles and then sealed with wax. In set No. 2 the seeds were preserved in desiccators in which the air was replaced by nitrogen freed from oxygen by passing through pyrogallol solution and then drying by passing through a tower of calcium chloride. In set No. 3 the seeds were taken in 15 cm × 2.5 cm test tubes and completely immersed in sterile liquid paraffin as is used in the preservation of fungal subcultures and then corked. The three sets were stored for 6–9 months in room temperature and then taken out for testing viability in the usual way. Samples from set Nos. 1 and 2 were used straight for germination tests. The seeds from set 3 were taken out, excess paraffin was drained out and then the seeds rinsed quickly with three changes of alcohol-benzene mixture (1 : 2) to remove the residual paraffin. The seeds were then spread on a petri dish to allow the alcohol-benzene mixture to evaporate and thereafter used for testing viability. The results of the three sets are given in Table I, where it is seen that the viability of the seeds under all the three conditions of storage was practically unaffected.

From the results of set No. 1, it is perfectly clear that jute seeds can be stored in tightly packed condition and there is no compelling need to leave any empty space. Results of set No. 2 show that oxygen is not necessary for jute needs during storage. But the condition of the experiment is open to the criticism that the oxygen of the desiccators had not been fully replaced by oxygen-free nitrogen. This point is, however, met by the

TABLE I  
Storage of jute seeds in the absence of oxygen

Set No.	Condition of storage	Period of storage	Percentage of germination		Vigour of germination	
			JRO-632	JRC-321	JRO-632	JRC-321
Control	..	..	97	98	Normal	Normal
1	Tightly packed in glass bottles	Six months	95	95	"	"
2	In desiccators in oxygen free dry nitrogen	..	95	96	"	"
3	Under liquid paraffin	Nine months	93	95	"	"

results of set No. 3. At the same time, it should be noted that *small* decreases in viability have appeared in sets 1, 2 and 3 as against the control. It remains to be seen whether these differences are significant and get magnified on prolonged storage. Further work is in progress.

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### X-RAY INDUCED CHANGES IN THE ACTIVITIES OF ACID AND ALKALINE PHOSPHATASE IN *PERIPLANETA AMERICANA*

CONSIDERABLE work exists to indicate the inactivation of enzymes following irradiation<sup>1-3</sup>. However, very little information is available on the sensitivity of enzymes to x-irradiation with respect

to sex and tissue of the animal, in which the enzyme occurs.

In the present investigation adult cockroaches of either sex were subjected to whole body exposure of x-rays, at doses ranging from 1,200 to 9,600 rads. Following the exposure, the activity of acid and alkaline phosphatase, both in the midgut and hepatic caeca, was determined as described by Bodansky<sup>4</sup>.

### RESULTS AND DISCUSSION

Both the enzymes showed a decrease in activity with the increase in the dosage of x-rays. The acid enzyme activity was completely suppressed at 4,800 rads in the male hepatic caeca and in the female midgut and at 6,000 rads in the male midgut and the female hepatic caeca. However, alkaline enzyme in both the tissues, irrespective of the sex of the insect, exhibited some activity (Table I), even after an exposure a dose of 9,600 rads.

These observations clearly indicate that acid phosphatase, irrespective of the insect's sex, is more sensitive to x-irradiation than alkaline phosphatase. But, its sensitivity to radiation varies in the two tissues studied and also with respect to sex of the insect. Acid phosphatase was more sensitive to

TABLE I  
Effect of x-rays on the activity\* of acid and alkaline phosphatase in cockroaches

Dose in rads	Male cockroaches				Female cockroaches			
	Acid enzyme		Alkaline enzyme		Acid enzyme		Alkaline enzyme	
	Midgut	H. Caeca	Midgut	H. Caeca	Midgut	H. Caeca	Midgut	H. Caeca
Normal	50.3	7.1	62.8	37.5	22.8	12.5	101.8	64.1
1200	36.8	5.1	50.7	21.4	10.3	7.6	82.1	39.3
2400	16.4	2.3	37.8	12.8	8.9	4.3	62.5	26.8
3600	11.9	1.1	30.7	8.4	5.0	0.7	35.5	13.2
4800	1.8	..	21.8	4.6	..	0.3	19.8	7.8
6000	..	..	15.3	3.6	..	..	13.2	5.7
7200	..	..	9.6	1.2	..	..	9.6	2.4
8400	..	..	6.1	1.1	..	..	7.1	1.2
9600	..	..	2.1	0.9	..	..	2.7	0.4

\* Enzyme activity expressed as  $\mu$ g of inorganic phosphorus liberated/ml homogenate/hour incubation.

radiations in the hepatic caeca of males and midguts of females.

However, the loss in activity of the enzymes following irradiation may be due to denaturation<sup>5</sup>, rupture of peptide bonds,<sup>6</sup> reduction of s-s- bridges as observed in acid phosphatase<sup>1</sup> or due to the effect of free radicals on the enzymes<sup>2,3</sup>.

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#### INFLUENCE OF ETHEPHON (2-CHLORO ETHYL PHOSPHONIC ACID) ON THE ACID PHOSPHATASE ACTIVITY IN THE SHOOTS OF TOMATO AND SWEET POTATO

INHIBITION of terminal growth and induction of lateral branching in plants by ethephon have been demonstrated in a wide range of plants (De Wilde, 1971). This could have been caused by many factors. El Fouly (1972) has suggested that the inhibition might be brought about by an increase in the activity of acid phosphatase.

In the present study, we investigated the effects of ethephon on the acid phosphatase activity. Sweet potato (*Ipomoea batatas* Cv. V6) and tomato (*Lycopersicon esculentum* Cv. Co. 1) were utilized for the study. Aqueous solutions of ethephon at 100, 250, 500 and 1000 ppm were sprayed on the foliage of 15 days old plants at the rate of 20 ml per plant using Teepol 0.1% as wetting agent. Samples of shoot tips containing 3 leaves were collected on next day of treatment for enzyme assay. The samples were ground in a homogenizer with 0.1 M sodium acetate buffer (pH 5.0) and centrifuged at 30,000 g for 30 minutes at 2° C. The supernatant solution was used as an enzyme source.

Enzyme reaction mixtures containing 0.3 mg of p-nitrophenyl phosphate and enzyme (100 mg fresh

weight equivalent) in 0.1 M acetate buffer (pH 5) to a final volume of 5 ml, were incubated in a shaker at 40° C (Young, 1965). Reaction mixtures were drawn at 5 minutes intervals for 30 minutes and enzyme inactivated with 15% trichloro-acetic acid. The phosphorus content of the mixture was estimated by the method of Fiske and Subbarow (1925) and the phosphatase activity was expressed as the amount of P/min/g of fresh wt. The total P content of the shoot tips was also estimated and is presented in Table I.

TABLE I

*Effect of ethephon on P and acid phosphatase activity in tomato and sweet potato shoot tips*

Ethephon (ppm)	Percentage of P (dry wt.)		Acid phosphatase activity mg P liberated/ min/g fresh wt.	
	Tomato	Sweet potato	Tomato	Sweet potato
Control	0.58	1.15	5.7	14.2
100	0.35	1.75	9.0	16.5
250	0.35	1.96	12.5	20.2
500	0.58	2.83	13.5	29.9
1000	1.15	2.04	7.5	13.7

It is clear from the data that ethephon stimulated the activity of acid phosphatase in tomato and sweet potato. The effect was accentuated with increase in the concentration of ethephon. However, a low activity was observed in 1000 ppm ethephon, but not lower than the control. Though the pattern of changes in acid phosphatase activity in response to the concentration of ethephon are alike, the activity was high in sweet potato than in tomato.

In tomato, 100 and 250 ppm ethephon decreased the total P while there was no change at 500 ppm. But it is interesting to note that total P doubled in 1000 ppm compared to control. However, in the case of sweet potato, total P content was found to be affected in the same way as the acid phosphatase activity.

Pratt and Goeschl (1969) reported an increase in ATPase activity in the leaves and stems of tomato plants treated with ethylene. De-Leo and Sacher (1970) have also shown a close association between respiration, CO<sub>2</sub> evolution and acid phosphatase activity in banana, induced to ripen with ethephon. Increase in respiration is generally observed in ethylene or ethephon treated plants. Changes in the phosphatase activity due to different concentrations of ethephon observed in this study might be a result of a denovo enzyme synthesis or of an activation (El-fouly, 1972). The reduction in the activity in 1000 ppm is difficult to explain at present. Phosphatase might have increased the respiration of the terminal shoots in tomato and

sweet potato, which would ultimately result in an inhibition of terminal growth as is generally obtained in ethephon treated plants.

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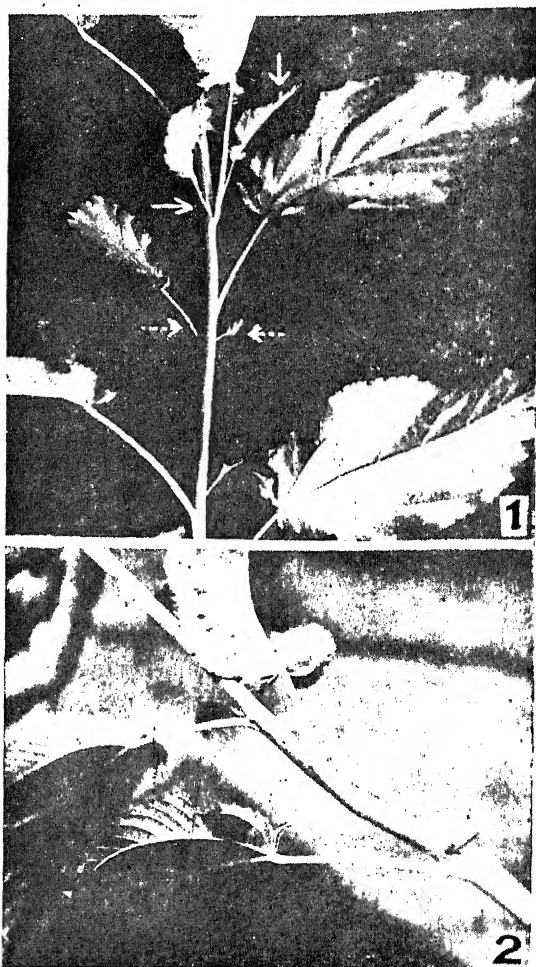
# FOLIACEOUS STIPULES—A NEW VARIANT OF OLITORIUS JUTE FROM INTERSPECIFIC HYBRID PROGENY

SWAMINATHAN AND IYER<sup>1</sup> have earlier reported the second successful interspecific  $F_1$  hybrid between grafted *Corchorus olitorius* var. JRO-620 and *C. capsularis* var. JRC-412 (pollen irradiated with 2 KR X-rays). The  $F_2$  and subsequent progenies of this cross showed a progressively increasing degree of skewness towards the female parent (*C. olitorius*). It was concluded by them that the two jute species have probably diverged towards an increased coherence of the parental genomes which automatically decreased the chances for effective recombination in the interspecific hybrid.

In a sustained search for possible recombinants in the succeeding generations, Iyer<sup>2,3</sup> located a single true-breeding line in the  $F_2$  progeny of the 1961 cross, which showed a distinctly new phenotype. The plants in this true breeding line were tall and luxuriant, and were marked by very broad leaves, possessing leaflike stipules at the two sides of the petiole, in addition to the normal filiform stipules. We have named this as the "Foliaceous stipule" variant and a brief report on this is presented here.

These plants were nearly green except for light red patches at the base and at the nodes. The leaves were much broader than those of the JRO-620 parent, with undulating surface and large overlapping serrations, the last pair of which were drawn into unusually broad appendages. The most striking feature, however, was the presence of well-developed leaf-like stipules, one on each side of the petiole, in addition to two other smaller stipules. Patel *et al.*<sup>4</sup> have reported the presence of foliaceous stipules in an exotic variety Halmehera of *C. capsularis*. Such an instance is so far un-

known in *C. olitorius*. When fully grown, these "Foliaceous stipules" were exactly like jute leaves, with a regular petiole and a lamina (Fig. 1) showing normal venations and appendages. In some nodes, we found only one leaf-like stipule and the other was simply foliaceous lacking a distinct petiole.



FIGS. 1-2. Fig. 1. A portion of vegetative shoot of the "Foliaceous stipule" variant, showing two fully grown petiolate, foliar stipules (—) in the upper nodes and, in the lower node, one of the two stipules is smaller (---). Note the undulating leaves with large appendages. Fig. 2. Part of a vegetative shoot of parent var. JRO-620 showing the normal pigmented, filiform stipules (—), one on either side of the leaf petiole.

From early stages of growth one can distinguish these accessory leafy stipules even in the 3 leaved seedling. Subsequently, these stipules expanded in size, undergoing curvatures due to unequal growth



of the two edges. Later, one on either side was transformed into a leaf. The peculiar feature was the absence of red pigment on the leafy stipules, but one could discern a minute dot-like red pigment spot at the base of the smallest of the 2 or 3 stipules. In contrast to this, the parental variety JRO-620 showed bright red, filiform stipules, one on either side of leaf-petiole (Fig. 2). Further, whereas the normal stipules were caducous dropping off as the leaf matured, those in the 'foliaceous' variant persisted till harvest time, even when the true leaves had senesced.

The above feature prompted us to check the variant's yielding ability and in preliminary trials, is proved superior to the parent variety but did not, however, exceed the yield of the standard JRO-632 variety. Nevertheless, in view of the unusual recessive marker available, we are incorporating it in our crossing programme for varietal improvement. Another redeeming feature of this hybrid was its freedom from mite attack and stem-rot. Cytologically these plants showed  $2n = 14$  chromosomes and normal seed fertility.

We have carried out extensive anatomical and genetic studies to understand the nature of these leafy stipules and the indications are that these structures represent accessory stipules that have got transformed into leaf-like structures. Being an entirely new character as yet unknown in the two parents of the cross, we believe this is either a transgressive segregant or a new recessive mutation whose origin could probably be traced to the use of X-irradiated pollen in the original cross, which had also resulted in trisomics<sup>5</sup> in the progeny. Details of these studies are being reported elsewhere.

We are grateful to the Director and Head, Division of Genetics, Indian Agricultural Research Institute, for providing facilities for this work, and to Dr. M. S. Swaminathan, F.R.S. and Prof. B. C. Kundu, F.N.I., for advice and encouragement. The excellent technical assistance of Sh. K. L. Gupta is gratefully acknowledged.

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### ALBOMACULATA TYPE OF VARIATION IN COIX

OUT of a large population of *Coix aquatica* Roxb. and *C. gigantea* Koen. raised during 1966–1972, eight plants, in different years, in *C. aquatica* and one plant in *C. gigantea* exhibited variegation in leaves and other parts of the plant body, in the form of narrow longitudinal white stripes in the former species and yellow stripes in the latter. Such striping, resulting from chlorophyll deficiencies, in several plant species, is known to show Mendelian segregations, while in many others it follows non-chromosomal inheritance<sup>1-7</sup>. The inheritance of the striped character in the two species is reported here.

In *C. aquatica*, when the striped plants were: (i) selfed, (ii) open pollinated, (iii) crossed with a green plant (as male) or (iv) mated to each other, the resulting progenies consisted mostly of green seedlings as the white lethals occurred only in a very low proportion. No striped plants were found in the offspring. When a green plant as female was crossed with a striped one as male, the progeny comprised only of green seedlings. The  $F_1$  green plant (striped  $\times$  green) produced only green offspring in  $F_2$  (Table I).

In *C. gigantea*, the striped plant when (i) selfed, (ii) crossed to a green plant, with the latter as male, or (iii) open pollinated, the progeny contained green, striped and yellow lethals in random frequencies. When the striped plant was crossed as male with a green one, the progenies were all green (Table I).

Both yellow and white lethals died at the two leaf stage. The striped plants in either species did not show any aberrant meiotic behaviour. Further information on their breeding behaviour could not be obtained as striped plants did not appear in the progenies in *C. aquatica* and the few that came up in the offspring in *C. gigantea* died before flowering. The fact that the striped plant did not breed true for the character on selfing, that the progenies of the reciprocal crosses were not identical and that the trait appeared in the progeny, in *C. gigantea*, only when the striped plant was used as seed parent, suggest that the character is not transmitted either through nuclear genes or male parent. Further, the fact that some of the tillers that arose subsequently in the striped plant were entirely green indicates somatic segregation of green and white or yellow cells. Variegation, having generally the characteristic of apparent gene independence, maternal inheritance and random distribution of green and white (or yellow) cells, that has been described in many a plant species, is known as *status albomaculatus* after Correns<sup>1</sup>, the trait being transmitted through the cytoplasm

TABLE I

Progenies from self-pollinated, open-pollinated crosses between striped and green parents of  
*C. aquatica* and *C. gigantea*

Pollinations	Seedling progeny			
	Green	Striped	Yellow or white lethals	Total
<i>C. aquatica</i>				
1. Striped plant self-pollinated ..	78	..	1	79
2. Striped plant × Green plant (F <sub>1</sub> ) ..	102	..	1	103
3. Striped plant open pollinated ..	166	..	2	168
4. Striped plant × Striped plant ..	92	..	1	93
5. F <sub>1</sub> green plant (from item 2 above) self-pollinated (F <sub>2</sub> ) ..	75	..	..	75
6. Green plant × Striped plant (F <sub>1</sub> ) ..	118	..	..	118
<i>C. gigantea</i>				
1. Five tillers of a Striped plant self-pollinated ..	129	1	4	134
2. Three tillers of the same Striped plant × Green plant ..	71	1	3	75
3. Green plant × Striped plant ..	148	..	..	148
4. Two tillers from the Striped plant × Green tillers from the same striped plant ..	61	1	2	64

or plastids of the egg. Accordingly the inheritance of striping, in the present study, may be explained on the basis that, irrespective of the male parent, zygotes formed on the green portions of the plant contain green plastids and produce only green offspring, those borne on the yellow or white portions have yellow or colourless plastids and give rise to only yellow or white lethals, and those developed on the adjoining regions of yellow and green have both yellow and green plastids and produce striped seedlings in the progeny. Since the proportions of white or yellow regions, compared to green areas, in the striped parents were much smaller, the frequency of white or yellow lethals obtained in the progenies was also far less.

My thanks are due to Professor J. Venkateswarlu under whose supervision this work was carried out.

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# KCl-INDUCED INCIPIENT PLASMOLYSIS AND ITS ROLE IN STOMATAL REGULATION OF *CONVOLVULUS MICROPHYLLUS* SIEB. EX SPRENG.

A LARGE number of theories have been proposed by various workers for stomatal regulation<sup>1-3</sup>. Active uptake of cations like Li, Na, K, Rb by guard cells is stated to be responsible for stomatal opening<sup>4</sup>. The role of starch sugar regulation in the guard cells has been considered to be an important cause of stomatal opening<sup>5</sup>, caused by the influx of potassium ions<sup>5-8</sup>. Plasmolysis of the epidermal cells has also been shown to be the cause of stomatal opening<sup>5</sup>. It has been shown that ions are taken up directly from the solution which accumulate in guard cells by the active uptake mechanism in the detached epidermis in respect to certain solution in the medium<sup>2,9</sup>. However, it is generally agreed that stomatal movement is brought about by the changes in the turgor of the guard cells<sup>1</sup>, and of the neighbouring epidermal cells<sup>10</sup>, and that these changes may be caused by osmotic gradients between guard cells and neighbouring tissue.

During the course of the investigation on water relations of some arid zone plants, interesting cases of stomatal regulation with possible uptake of cations from the incubating media were observed. The same is reported here for isolated epidermal peelings of *Convolvulus microphyllus*.

Epidermal peelings from the leaves of *C. microphyllus* were incubated in different concentrations

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(.4 M to .9 M) of KCl for a period of 1-3 hours and their control in the distilled water after the preliminary trials with 0.1 M to 1.0 M have already been made. After incubation period, the epidermal peelings were stained with neutral red to observe plasmolysis, if any. Stomatal pore width was measured with precalibrated microscope. Stomata were very slightly open initially, but after one hour of incubation period, a maximum opening was observed in peelings incubated in .5 M KCl (Table I). It was interesting to note that in the above concentration, subsidiary/epidermal cells showed incipient plasmolysis (Fig. 1). In the increasing

TABLE I

Effect of an incubation period of 1 hour in different concentrations of KCl on stomatal pore width (in  $\mu$ ) and plasmolysis of subsidiary cells/epidermal cells in isolated epidermal peelings of *C. microphyllus*

Conc. in molar	Stomatal pore width	Plasmolysis in subsidiary/epidermal cells
0.0	2.7 $\pm$ 0.9	—
0.4	4.5 $\pm$ 2.2	—
0.5	11.1 $\pm$ 1.2	Incipient
0.6	2.1 $\pm$ 2.4	+
0.7	1.6 $\pm$ 1.4	+
0.8	0.9 $\pm$ 0.7	+
0.9	Close	+

Further work is in progress.

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#### NEW RECORD OF PARASITIC LARVAE OF *AGAMERMIS DECAUDATA* COBB ET AL., 1923 (NEMATODA: MERMITHIDAE) IN INDIA

DURING the general survey of Plant Parasitic nematode prevailing in the vicinity of Udaipur and its suburbs, authors observed preparasitic larval population of *Agamermis decaudata* Cobb et al., 1923 in a soil sample brought from a citrus orchard. These larvae have been reported parasitic on grasshoppers *Conocephalus brevipenne* and *Melanoplus femuncubrum* in U.S.A.<sup>2</sup>. Since to our knowledge the presence of *Agamermis* sp. has not been previously recorded from India this report forms the basis of first record. Beside this the nematode ranges its geographical distribution to India as well. According to a review article its geographical distribution was confined to U.S.A. only<sup>3</sup>. The dimension and short description is provided and illustrated.



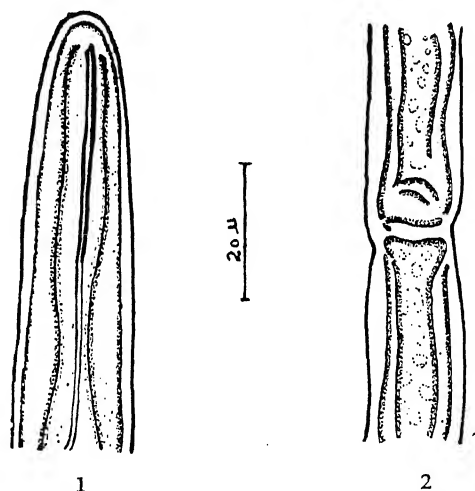
FIGS. 1-2. Fig. 1. Epidermal peeling incubated in 0.5 M KCl showing maximum stomatal opening at incipient plasmolysis in *C. microphyllus*,  $\times 850$ . Fig. 2. Epidermal peeling incubated in 0.9 M KCl with distinctly plasmolysed subsidiary and guard cells showing closed stomata in *C. microphyllus*,  $\times 850$ .

concentrations, the plasmolysis of the subsidiary/epidermal cells also increased. With the increasing plasmolysis, the stomatal opening got reduced significantly (Table I). In peelings incubated in .9 M KCl, distinct rounded plasmolysed contents were observed in the subsidiary cells. Guard cells also appeared to be plasmolysed and stomata completely closed down (Fig. 2, Table I).

Dimension :

$L = 1.35 \text{ mm}$ ,  $a = 192.9$ ,  $b = 3.9$ ,  $c = ?$   
Stylet =  $30 \mu$ , Node =  $.037 \text{ mm}$  (From the anterior region).

The larvae are creamy white in colour, long slender, possess a tapering proximal and a blunt distal end. The mouth leads directly to pharynx (Fig. 1). Oesophagus long devoid of musculature. Stylet dorylaimoid. The node (Fig. 2) is clearly distinguishable from the rest of the body, from where larval tail amputates as soon as the larvae invades its host.



FIGS. 1-2. Anterior region and Node of *A. decaudata* larvae, ( $15 \times 40$ ).

Authors are indebted to Dr. P. A. A. Loof, The Netherlands, for identification; The Head, Division of Entomology, I.A.R.I., New Delhi, for pertinent information and to the Director, Agricultural Experiment Station, University of Udaipur, Udaipur, for providing necessary facilities.

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A NEW SPECIES OF *CEPHALOSPORIUM* FROM THE RHIZOSPHERE OF GROUNDNUT

WHILE studying the effects of agronomic treatments on the changes in the rhizosphere mycoflora of groundnut, var. SB-11, a species of *Cephalosporium* was isolated by soil dilution plate method using Waksman's synthetic agar medium. Its morphology was compared with the species of *Cephalosporium* described earlier by various workers<sup>1-6</sup>. The comparison clearly brought out the differences (Fig. 1),

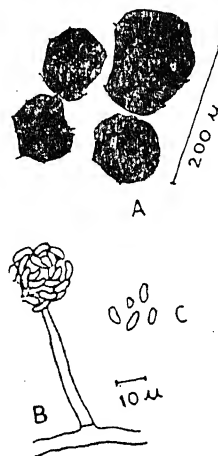


FIG. 1

especially in the presence of sclerotium, on the basis of which the isolate has been described as a new species of *Cephalosporium*, to be called *Cephalosporium sclerotiorum*.

*Cephalosporium sclerotiorum* Sp. Nov.

Colonies on potato dextrose agar attaining a diameter of about 3-4 cm in 10 days at room temperature ( $25 \pm 2^\circ \text{C}$ ), floccose, white at first, turn to black when sclerotia develop in circular zones, margin thin and diffuse, reverse gray; hyphae hyaline, creeping, unseptate, branched,  $1.5-2.0 \mu$  thick; conidiophores unbranched, septate at base,  $15-20 \times 1.8-2.0 \mu$ , bearing a head of conidia enclosed in slime; heads commonly  $5-10 \mu$  in diameter, bearing 7-20 conidia in each; conidia hyaline, elliptical straight or slightly curved, sometimes globose, thin walled, smooth, with one end slightly tapered, ranging  $2.5-7.0 \times 1.5-2.0 \mu$ ; sclerotia dark black, spherical, ovate or irregular,  $40-200 \mu$  in diameter.

*Cephalosporium sclerotiorum* Sp. Nov.

Coloniae supra PDA in  $25 \pm 2^\circ \text{C}$  temp., intra dies 10, 3-4 cm diam. evadentes, floccose, primo albae, dein nigrae, marginibus tenuibus et diffusis, reverso griseae, cum productione sclerotiorum in

zonis circularibus; hyphae hyalinae, repentes, non-septatae, ramosae, 1.5–2.0  $\mu$  crassitudine. Conidiophora nonramosa, versus basim septata, caput conidiorum in muco immersum portantia. Capita generatim, 5–10 diam., singillatim 7–20 conidia exhibentia. Conidia hyaline, elliptica, parietibus tenuibus, laevia, ad unum terminum paulo angustata, 2.5–7.0  $\times$  1.5–2.0  $\mu$ . Sclerotia atra, spherica, ovata vel irregularis, 40–200  $\mu$  diam.

We thank to Rev. Fr. Dr. C. Saldhana, St. Joseph's College of Science, Bangalore, for Latin diagnosis of the species.

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#### INDUCED MULTILOCLAR Ovary IN *CAPSICUM ANNUM* BY X-RAYS

THE ionizing radiation has played a main role in inducing mutations in plants and many investigations have been carried out in the direction of induction by various workers<sup>1</sup>.

To our knowledge, however, no report is available to indicate the modification of the locules of ovary due to ionizing radiation in *Capsicum annum*, L.

This paper presents results demonstrating a significant increase in the number of "carpels and locules" per ovary following X-ray irradiation on the dry seeds of *Capsicum annum*.

The seeds of NP 46 A were obtained from National Seed Corporation of India, Warangal Branch, and were subjected to X-ray irradiation at the Radium and Cancer Research Institute, Hyderabad. Various doses were applied and the initial dose was 1,000 rads.

In order to study the morphological variations, the irradiated seeds were sown in experimental plots.

The seeds irradiated with the X-rays produced elongated stems and other morphological variations such as increased branches, petals and stamens. Significant increase in the number of locules 6 (Fig. 1) per ovary were observed in the flowers following irradiation as compared with the controls, which have only two locules (Fig. 2).

Such mutants were observed at 2000 rads and 5% of the total plants were isolated in M-II generation. In addition to the increase in the number of carpels and locules of ovary, the diameter of irradiated flower was larger than that of controls, but the number of flowers was reduced significantly.

The thickness of the placenta increased. The formation of ridges on the surface of the fruit was an important character observed during the investigation.

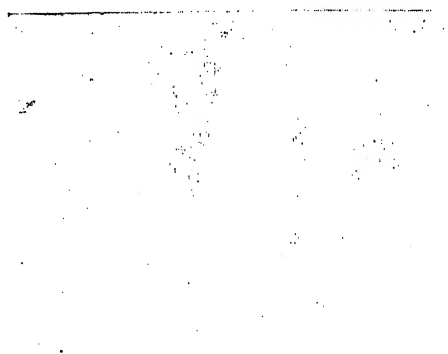


FIG. 1. Showing the number of locules of irradiated.

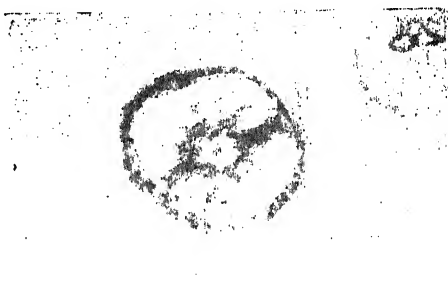


FIG. 2. Showing the number of locules of controls.

The increased locule number is correlated with cytological variations. The meiotic division in M-I and M-II generations showed great abnormalities such as anaphase bridges, and fragments.

Preliminary observation indicated that the mature fruits from flowers with radiation induced multilocular ovaries were significantly larger than the mature fruits from controls. The available evidence strongly supports the suggestion of a relationship between locule number and size of the fruit of chillies. Since the fruit size, an important economic character is usually correlated with the increased locule number a knowledge of the morphogenetic basis of the character seems worthwhile.

The findings raise the possibility that radiation induction of locule number in *C. annuum* is possible and if used in conjunction with other genetic and cultural techniques could prove to be of practical significance.

We gratefully acknowledge the financial assistance (to K. Subhash) of University Grants Commission for the Research.

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# **CYANEOLYTIA ACTEON (LAPORTE) (COLEOPTERA: MELOIDAE) A NEW PEST OF MAIZE AND BAJRA**

IN village Jaitpur (near Hoshiarpur, Punjab) maize and bajra (*Pennisetum typhoides*) leaves were noticed being eaten by a beetle which was later identified as *Cyaneolytta acteon* (Laporte) with the

Some preliminary observations on the biology of this pest were made. In the last week of June (1972) the adults were seen mating in the field and this act lasted 3-4 minutes. The males of this shining black beetle were 24 mm long and carried one bright red spot on each of the elytron whereas the female was 30 mm long having two spots on either side. The females were observed to lay elongated yellowish eggs in clusters on the soil surface or on the upper side of maize or bajra leaves. A female on an average laid 123 eggs. The egg stage lasted 2.6 days in July and 87% of them were viable. The newly hatched triungulins were very active to start with and lived for 4.9 days without feeding either on leaves or roots of the host plants. As to how the pest survives in the immature stages under field conditions needs to be studied.

This appears to be the first record of the insect as a pest of maize and bajra in India. Fletcher (1921) has reported it as occurring on grass, lucerne, rice, *Setaria* and *Panicum miliaceum* at Pusa and also the occurrence of the blister beetles *Lytta tenuicollis* Pall., *L. picta* Cast. and *L. ruficollis* Oliv. on bajra. In Karnataka State it has been noticed to infest rice, sorghum and *Eleusine coracana* (Usman and Puttarudraiah, 1955).

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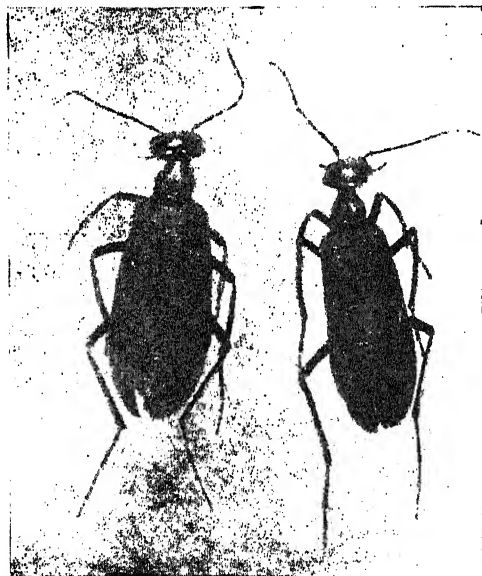
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# **A NEW SPECIES OF ACROSTAPHYLUS FROM PHYLLOSPHERE OF BRASSICA JUNCEA**

THE genus *Nodulisporium* Preuss, widely recognized as the *status conidialis* of Xylariaceae, was erected for two moniliaceous fungi, *N. album* Preuss and *N. ochraceum* Preuss. Since then a number of other species of this genus have been described<sup>6-8,3,4,2</sup>. Arnaud<sup>1</sup> erected the new genus *Acrostaphylus* Arnaud for *Nodulisporium* like dematiaceous fungi. On this basis, Subramanian<sup>9</sup> recommended that the name *Nodulisporium* be exclusively used for moniliaceous and *Acrostaphylus* for dematiaceous types. Rogers<sup>5</sup> on the basis of conidiophore and conidial colour differences influenced by age, environment, etc., chose not to assign the conidial *Hypoxylon fuscum* Pers. ex Fr. to either genera.

This paper describes a new species of *Acrostaphylus*, *A. ornatus*, referred to this genus on the basis of its dematiaceous appearance. The fungus

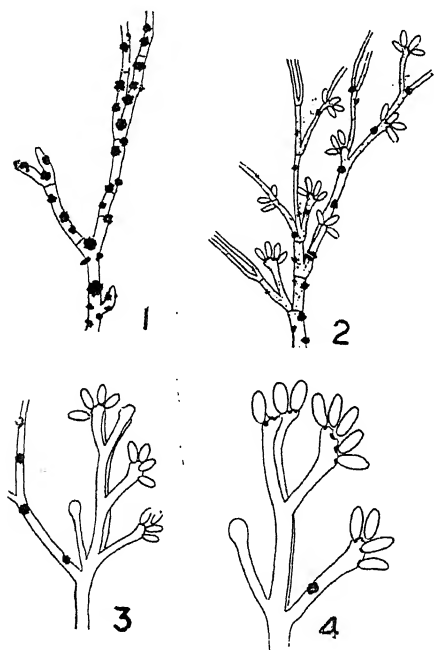


assistance of the Director, Zoological Survey of India, Calcutta. The density of 52 beetles in 4 square meter area was quite high and the damage was noticeable. Two pairs of beetles when given 10-day old maize and bajra plants in the laboratory ate them up in 21.8 and 18.0 hours respectively, indicating that they are voracious feeders.

shows its close resemblance to *Acrostaphylus africanus* in colony characters with thickly studded dark-coloured basal hyphae and conidiophores, but differs from that in the branching of conidiophores and size of conidia which are considerably larger in the new species.

*Acrostaphylus ornatus* SPEC. NOV. (Figs. 1-4)

Coloniae crescentes cito in agar Czapek-Dox ad 30° C, primo albidae, tum fuscogriseae vel fere nigrae, constantes e mycelio floccoso aereo at serie basali stromatica crassa. Conidiophora primo hyalina, tum fuscobrunnea, fortiter incrustata granulia alta rubrobrunneis, irregular, iter vel sympodice ramosa, ornata at apices terminates moderate inflatos catervis comdiorum, septata 216.0-360.0  $\mu \times$  3.3-4.8  $\mu$ . Conidia insidentia projectionibus denti similibus, pallide brunnea, anguste pyriformia, basi truncata 6.8-11.2  $\mu \times$  3.4-4.2  $\mu$ .



FIGS. 1-4. *Acrostaphylus ornatus* spec. nov. on Czapek-Dox agar. Fig. 1. Basal hyphae showing ornamentation,  $\times$  315. Figs. 2-3. Showing variously branched and studded conidiophores. Fig. 2,  $\times$  195; Fig. 3,  $\times$  225. Fig. 4. Conidiophore tips showing attachment of conidia,  $\times$  825.

Lectuo e phyllosphaera *Brassicae juncea* mense decembri, 1967, ad Lucknow in India. Cultura posita No. CMI ad Kew IMI 129796.

Colonies on Czapek-Dox agar fast growing, initially whitish then becoming dark grey to almost black, forming a compact brownish black stromatic layer on the substratum and floccose pale to light grey aerial mycelium which bears abundant fruiting structures. Basal hyphae olivaceous brown, 5.1-6.8  $\mu$  in diameter, closely septate forming a complex stromatic layer, thickly studded with dark coloured wart-like excrescences; conidiophores erect or slightly curved, 216.0-360.0  $\mu \times$  3.2-4.8  $\mu$ ; lateral branches copiously 3-8 times branched irregularly, sympodially or sometimes into terminal verticils measuring 12.8-128.0  $\mu \times$  3.2-3.5  $\mu$ , hyaline at first, becoming dark brown with age, thickly studded with dart-like structures as in case of basal hyphae. Conidia light brownish in colour, elongate elliptical or fusoid, smooth, with a truncate base 6.8-11.2  $\mu \times$  3.4-4.2  $\mu$  borne singly on denticles of the flattened apices of conidiophores, initially by 'ballooning' of the thin apical portion, subsequently becoming lateral through elongation of the conidiophore apex or the sporogenous cell, producing a cluster of several conidia, the denticles remaining persistent.

Thanks are due to Dr. G. C. Ainsworth and Dr. M. B. Ellis for their valuable comments on the species.

Dept. of Botany,  
Lucknow University,  
Lucknow, May 31, 1974.

J. N. RAI.  
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## SHORT SCIENTIFIC NOTES

### A New Record of Wilt of Gram Caused by *Fusarium solani*

*Cicer arietinum* L., commonly known as gram, Bengalgram or chickpea, is grown over an area of about 8 million hectares in India. During 1970-74 the authors observed several gram fields severely infected with wilt disease in the states of Haryana and Punjab as well as in villages around Delhi. Initially diseased plants get stunted, leaves turn yellow and are ultimately killed. Infected plants were examined and found to be infected at root region. In thin sections of the infected roots, mycelium was invariably observed in the disintegrated cortex region and xylem vessels.

The fungus was isolated from the roots of infected plants on potato dextrose agar. In some severely infected plants, fungus was also isolated from the collar region, one inch above the ground level. The fungus was purified by taking one single cell spore and identified as *Fusarium solani* (Mart.) Sacc.

Colonies on potato dextrose agar, at first white to smokegrey (Ridg., 28A2) turning at maturity to lichen green (Ridg., 26A4). Colonies on oat meal agar, at first Italian straw colour (Ridg., 11D2) turned at maturity Carydalis to chrysolate green, due to formation of sporodochia. Conidia scattered with false heads. Sporodochia or pionnotes in a group and embedded in stroma. Stroma leathery, green in colour.

In order to test the pathogenicity of *F. solani*, 90 days old healthy plants grown in pots on sterilized soil, were inoculated by removing the soil around the plant upto two inch depth, and replacing it with 50 g of sand maize meal medium infested with *F. solani*. In controls sand maize meal medium without fungus was used to replace the soil. All inoculated plants developed disease symptoms which resembled with the symptoms observed in the field. Most of the inoculated plants were killed within three weeks. Reisolation from such infected plants yielded *F. solani* which resembled the original inoculated culture. Controls, however, remained free from disease. Pathogenicity of *F. solani* was reconfirmed over a period of two years.

There are reports by Chattopadhyay and Sengupta (1955), Chattopadhyay and Basu (1957), Bakshi and Singh (1959) as well as Bose and Sengupta (1961) of *F. solani* causing wilt in *Psidium guajava*, *Abelmoschus esculentus*, *Delvergia sissoo* and *Enterolobium saman* respectively. Kerr (1963) reported

root rot and *Fusarium* wilt complex of pea to be caused by *Fusarium solani* f. sp. *pisi*.

The authors are grateful to Dr. C. Booth, C.M.I., Kew, Surrey, England, for confirming the identity of *F. solani* and to Dr. S. P. Raychaudhuri for providing necessary research facilities for work.

Division of Mycology and J. S. GREWAL.  
Plant Pathology, MAHENDRA PAL.  
Indian Agricultural Research D. D. KULSHRESTHA.  
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### New Record of *Pheidole* sp. (Hymenoptera: Formicidae) as a Predator of the Rice Leaf Folder *Cnaphalocrocis medinalis* Guen.

In several parts of Kerala, larvae of *Cnaphalocrocis medinalis* were found attacked by adults of *Pheidole* sp. The activity of the predatory ants was maximum in upland crops where they could reach the leaf folds easily. They were also observed in wet lands reaching the plants through floating materials.

Workers and soldiers of *Pheidole* sp., collected from field bunds, were separately confined in groups of twenty along with *C. medinalis* larvae in varying numbers in deep petri dishes containing loose soil for studying their feeding potential and predatory habits. Workers were more active than soldiers and preferred the third and fourth instar larvae of the pest. On an average each group of twenty workers rendered upto fifteen larvae moribund within a period of 15 minutes. A few of the cadavers were dragged into the soil and fed upon, while the remaining ones were left on the soil. Thus they were killing more number of larvae than actually required for their feeding.

*Pheidole* spp. have been previously reported as predators of the larvae of *Sesamia inferens* (Yanagihara, 1934) and of *Carpocapsa pomonella*



(Jaynes and Marucci, 1947). They were also seen feeding on the eggs of *Theraptus* sp. (Tait, 1954) and *Oncopera intricata* (Martyn, 1965).

The authors express their gratefulness to the Director, Commonwealth Institute of Entomology, London, for the help rendered in identifying the predator.

Division of Entomology, N. M. DAS.  
College of Agriculture, C. C. ABRAHAM.  
Vellayani, Kerala, KUNJAMMA P. MATHEW.  
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#### A New Family of Chalcidoidea (Insecta: Hymenoptera) *EURYISCHIDAE* fam.n.

The present writer proposes a new family, Euryischidae, based on the genus *Euryischia* Riley. This genus possesses certain special characters which refrain it from falling in any of the known families of Chalcidoidea. In keys to the families of Chalcidoidea, proposed by Mani (1938), Nikol'skaya (1952), Brues *et al.* (1954) and Peck *et al.* (1964), the genus *Euryischia* runs near the family Elasmidae. However, it differs from this family in having 5-jointed tarsi; post-axillae; thorax with complete parapsidal furrows; fore wings with well-developed submarginal, marginal, postmarginal and stigmal veins; and tridentate mandibles.

In Vierek's (1916) and Essig's (1954) keys to the families of Chalcidoidea, the genus *Euryischia* runs near the family Aphelinidae. However, it differs from this family in having post-axillae; much enlarged propodeum; much compressed, and disc-like hind coxae; and two long and thick spurs at apex of hind tibiae.

In Comstock's (1954), Imms (1957), Borror and Delong (1963) keys to the families of Chalcidoidea, the genus *Euryischia* neither falls in the family Elasmidae nor in Aphelinidae due to having some special characters for which a new family Euryischidae is proposed.

The new family Euryischidae is characterised as follows:

Mandibles tridentate; antennae 8-segmented excluding the ring segments; thorax with complete parapsidal furrows; post axillae present; propodeum much enlarged; fore wings with well-developed submarginal, marginal, postmarginal and stigmal veins; hind coxae much compressed and disc-like;

hind tibiae with two long and thick spurs at apex; tarsi 5-jointed; abdomen longer than thorax, flat above and keeled below.

Type-genus, *Euryischia* Riley.

The present writer is greatly indebted to Prof. S. Mashhood Alam, Department of Zoology, under whose guidance this work was carried out. He is also thankful to Prof. Nawab H. Khan and Dr. Man Mohan Agarwal for encouragement.

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#### New Record of *Choribus* sp. (Hymenoptera: Braconidae) as Parasite of the Rice Whorl Maggot *Hydrellia philippina* Ferino

The rice whorl maggot *Hydrellia philippina* (Diptera: Ephydriidae) has been recorded as a pest of rice seedlings in Kerala causing substantial damage to the autumn crop grown from April-May to September-October (Thomas *et al.*, 1971).

While rearing the pest collected periodically from different parts of the State, *Choribus* sp. (Braconidae) was recorded as a solitary endoparasite of the pupal stage. The seasonal peak in the parasite population was in July, the maximum parasitism being 3%.

This is the first record of the parasite on *H. philippina*. *Choribus aquaticus* Mues. has been



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## REVIEWS AND NOTICES OF BOOKS

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Evolution of the Genus *Homo*. By William Howells. (Addison-Wesley Publishing Company), 1973. Pp. 188.

How old is Man? Indeed, what do we mean by MAN? When did an ape become "human"? If upright walk is of man's distinctive character, it is probably easy enough to say when such a man emerged. But there is more. Man is so distinctly different from all other anthropoids, and we have to rely only on palaeontology for evidences of his emergence from the apes, that it is difficult to establish when this emergence occurred, even at the million year level, which is permissible in palaeontology. That Man is also the investigator as well as the subject of investigation adds enormous interest, as well as controversy, to the subject.

It is now over eighty years since the first really primitive human fossil was discovered in Java by Eugene Dubois, who named it *Pithecanthropus erectus* and since then several early fossils of man have been added. But only since the Leakey family's discoveries in East Africa has it been possible to put together anything like a connected account of the early history of Man and his subsequent evolution.

There is now fair agreement that the middle and upper Pleistocene was the scene of Man's origin, and that the large brain characteristic of later man is found in the fossils of the upper Pleistocene. It also seems clear that hominid evolution occurred in essentially two successive stages as species, *Homo erectus* of the middle Pleistocene and *Homo sapiens* of the upper Pleistocene. *Ramapithecus* represents perhaps the first shift from the pre-hominid to the hominid condition but he was not Man. *Ramapithecus* was essentially a tree-liver with broad shoulders and chest, a stocky body and a lumbar spine. Diet is a true test of human evolution and the form, length and shape of the jaws and the teeth are fairly good indices of this. *Ramapithecus* had a short jaw with diminished fore-teeth and heavy molars for grinding. To that extent he was

different from the modern apes, whose front teeth are prominent features of their jaws.

But *Ramapithecus* had a small brain, and large brain capacity is characteristic of *Homo*, both of *erectus* as well as of *Sapien*. Unquestionably this has relation with speech and tool-making ability, though it is now recognized that not only size but also quality of the brain has bearings on later human evolution.

Howells presents a masterly yet cautious analysis of the existing data on human evolution which permits the incorporation of future discoveries. For evolution of *Homo* is a "hot" subject. It has already yielded several important facts: that ape-man separation took place about 14 million years ago; that it was characterized by a progressive increase in brain capacity; that vocal anatomy changed to permit develop speech and language and that the development of a cortex was a crucial step to achieve these. But at least two important questions remain. How did these significant differences between ape and man arise? How long did it take for them to develop?

Howells's book presents a clear summary of the present position in regard to human evolution. It is a good text-book for anthropologists and sociologists. B. R. S.

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### ANNOUNCEMENTS

#### Perspectives of Structure and Function of DNA

An International Symposium on "Perspectives of Structure and Function of DNA" will be held under the auspices of the Association of Microbiologists of India in Bangalore during December 22 and 23, 1974 to mark the Twenty-First anniversary of the publication of the double helical structure of DNA. Several leading scientists from both within our country and abroad are expected to participate in the symposium.

Intending participants should write to the General-Secretary, A.M.I., Department of Microbiology, M.S. University, Baroda 390 002.

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# DEPARTMENT OF SCIENCE AND TECHNOLOGY NEW DELHI

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davp 647(11)/74.

# SPECTROPHOTOMETRIC DETERMINATION OF ANTIMONY AND BISMUTH IN THEIR MIXTURE AFTER SEPARATION BY SOLVENT EXTRACTION AND PAPER CHROMATOGRAPHY

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## INTRODUCTION

**D**ETERMINATION of antimony and bismuth which usually accompany each other, mostly in biological materials, is of particular importance. McChesney<sup>1</sup> described a method for the determination of both in biological materials, using varying concentrations of the iodide ion. Bismuth, in presence of 200 p.p.m. or below of antimony, was determined by complexing antimony with fluoride or tartaric acid<sup>2</sup>. Besides, several other methods<sup>3-6</sup> are known for the determination of bismuth in samples containing antimony and other metal ions where bismuth was separated first by solvent extraction. West and Hamilton<sup>7</sup>, on the other hand, described a method for the separation of antimony from many elements by solvent extraction. Antimony in aqueous solution containing potassium iodide and sulphuric acid was extracted with benzene which was then identified with rhodamine B.

Based on this and also from the fact that antimony and bismuth can be determined separately by developing their colour with potassium iodide in sulphuric acid, an extraction spectrophotometric determination of both was tried.

Large number of investigators<sup>8-11</sup> have effected separation of many metal ions by paper chromatographic method and in many cases determined their  $R_f$  values. As an alternative to the extraction photometric method, chromatographic separation followed by spectrophotometric determination as their iodo complex was also studied.

## EXPERIMENTAL

**Reagent.**—An 11.2% solution of potassium iodide containing 2 gm per 100 ml ascorbic acid was used as chromogenic reagent.

**Standard Solutions.**—For the extraction method of separation, antimony and bismuth sulphate solutions were prepared in a mixture of dil. sulphuric and nitric acids. The resulting solutions was diluted to contain 100 ppm in each.

For separation by chromatographic method, a synthetic mixture of both in HCl containing 1000 ppm of each was prepared.

**A. Extraction spectrophotometric method.**—Synthetic mixtures of chloride free bismuth and antimony were prepared by taking aliquots contain-

ing 30, 25, 20, 15, 10 and 5  $\mu$ g of bismuth in 50 ml separating funnels and adding to these 5, 10, 15, 20, 25 and 30  $\mu$ g of antimony respectively. Each of these solutions were diluted to 5 ml by adding 0.75 ml of 27 N sulphuric acid (solutions should be 4 N with respect to sulphuric acid), 2.5 ml of potassium iodide reagent and distilled water. Solutions were allowed to stand for few minutes and then shaken with 5 ml portion of benzene for 5 minutes. Aqueous layers were transferred to another set of funnels and extractions, in this manner, were repeated 3–4 times. Combined benzene extracts, in each, was then evaporated on a water bath and the residue was treated with sulphuric acid and potassium iodide as above making the final volume 5 ml. The resulting solutions which gave clear spectrum of iodoantimonite ion (Fig. 1) were read at 425 nm.

Aqueous layers were slightly warmed on water bath to remove any dissolved benzene and finally volumes were made up in 5 ml standard flasks. Characteristic spectrum of the iodobismuthite ion (Fig. 1) was obtained in the aqueous layer. Extinctions were measured at 460 nm. The results, as calculated from the individual calibration curve, are shown in Table I.

TABLE I  
Extraction spectrophotometric determination of bismuth and antimony

Bismuth $\mu$ g			Antimony $\mu$ g		
Present	Found	% Error	Present	Found	% Error
30.0	29.9	0.4	5.0	4.3	14.0
25.0	24.9	0.4	10.0	9.1	9.0
20.0	20.0	0.0	15.0	14.0	6.6
15.0	14.8	1.3	20.0	19.2	4.0
10.0	9.8	2.0	25.0	23.8	4.8
5.0	5.0	0.0	30.0	28.6	4.6

**B. Spectrophotometric determination after separation by paper chromatography.**—Whatman No. 1 chromatographic paper was cut into strips 30  $\times$  2.5 cm. Equal volumes of mixture (containing 1000 ppm bismuth and antimony) were then applied (keeping the spot area to a minimum) to two chromastrips with the help of a micro pipette at a distance of about 5–6 cm from one end of the strip.

\* The late Professor of Chemistry, Gauhati University.

Paper strips, after drying the spot with a hair drier, were kept for an hour or two in air before subjecting to chromatographic development.

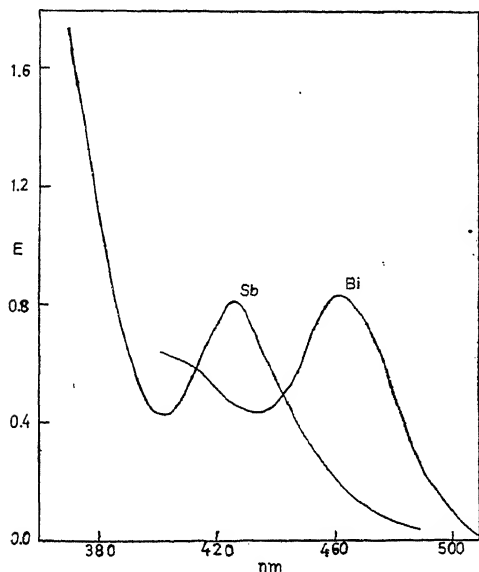


FIG. 1. Absorption spectra of Bi and Sb as their iodo complex after separation by solvent extraction. (Bi = 15  $\mu\text{g/ml}$ ; Sb = 20  $\mu\text{g/ml}$ ).

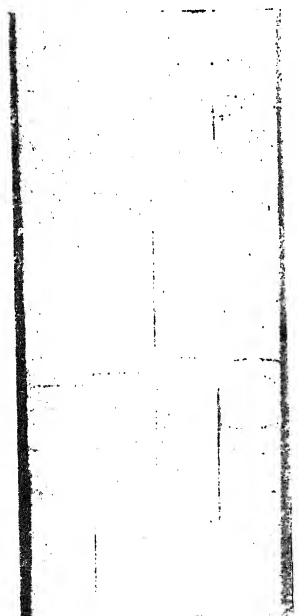


PLATE 1. Paper chromatographic separation of Bi and Sb. Upper spot—Sb; Lower spot—Bi.

The two strips (forming a pair) having equal amount of  $\text{Bi}^{3+}$  and  $\text{Sb}^{3+}$  were then fixed along a

glass "T" tube, keeping the spots in the two strips in parallel position. These strips were then lowered inside a cylinder of 6 cm diameter and chromatography was performed in an ascending manner using ethyl methyl ketone + 10% concentrated hydrochloric acid as developer. Mouth of the cylinder was closed with a rubber stopper. Development was allowed to continue for 5–6 hours till the solvent front moved to a distance of 20–25 cm. Paper strips were then taken out and dried, and one of the two strips (reference strip) was then sprayed with 0.5% oxine reagent, dried, and finally exposed to ammonia. A dark spot near the solvent front and a yellow spot below this (shown in Plate 1) was observed in this reference strip. These spots appeared as dark and purple respectively under ultraviolet light.

Corresponding regions from the unstained papers were then cut and put inside 50 ml conical flasks. These were then eluted with a mixture of 0.75 ml of 27 N sulphuric acid, 2.5 ml of potassium iodide reagent and 1.75 ml of distilled water. Solutions were allowed to stand for 5 minutes. Eluates of the first and the second spot gave characteristic spectra of antimony and bismuth respectively similar to the ones shown in Fig. 1. Their extinctions were measured at 425 nm and 460 nm and the amount of antimony and bismuth were read from the calibration curve. Results are shown in Table II.

TABLE II

*Determination of antimony and bismuth after paper chromatographic separation*

Antimony $\mu\text{g}$			Bismuth $\mu\text{g}$		
Present	Found	% Error	Present	Found	% Error
5.0	4.6	8	5.0	4.6	8
6.0	5.7	5	6.0	5.4	10
7.0	6.5	7	7.0	6.9	2
8.0	7.3	9	8.0	7.9	2
9.0	8.6	5	9.0	8.9	2
10.0	9.8	2	10.0	9.6	4

*Discussion.*—Of all the methods, the iodide method is the simplest and yet give satisfactory result. However, in their simultaneous determination, a double reading was necessary, once at 460 nm and then at 425 nm for bismuth using 1.6% potassium iodide reagent and another at 425 nm for total amount of bismuth and antimony using 11.2% potassium iodide reagent. Antimony was determined by subtraction after necessary correction for bismuth. In the present method 'A' the same concentration of the reagent is used and the determination is

carried out after separation by solvent extraction. Good recovery was achieved as shown by the average of a number of determinations in Table I. Antimony, could not be determined directly in the benzene layer as the layer showed faint iodine colour even in the reducing atmosphere of ascorbic acid.

Number of developer liquids like water, ethanol + 10% 5 N HCl, butanol + 10 N HCl, pyridine water were tried for chromatographic separation of bismuth and antimony. With the first two developers, both the metals formed a mixed spot. With butanol + 10 N HCl, although the separation was achieved, the spots eluted with KI did not give their characteristic spectra. Antimony and bismuth could be separated with pyridine-water as developer when applied as their nitrates. But when applied as chlorides, a mixed spot was obtained. However, high acid concentration in ethyl methyl ketone containing 10% concentrated HCl reduced the possibility of oxysalt formation and thus resulted in clean separation. The ketone

escaped on drying the paper and the spot eluted with KI gave characteristic spectra facilitating their spectrophotometric determination with an error less than 10% in the ppm range.

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## NUTRITIONAL EVALUATION OF SOME INDIAN NONCULTIVATED WILD LEGUMINOUS SEED PROTEIN ISOLATES

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The protein isolates from some uncultivated wild leguminous seeds when supplemented with the limiting amino acids, methionine and tryptophan and fed to animals, no untoward symptoms were noticed and the diets proved capable of promoting growth and maintained positive nitrogen balance in them. Also they did not appear to induce any deleterious physiological after-effects on the animals as evinced by liver protein and several liver enzyme assays.

IN a previous communication<sup>1</sup> a number of wild leguminous seeds were analysed for their chemical composition and their essential amino acid content. Finding their protein and amino acid contents only slightly inferior to casein it was considered pertinent to supplement them with the inadequate amino acids and test their efficiency as growth promoters. However, as their unpalatability, bad odour and toxicity in some cases disallowed feeding of the entire seeds to experimental animals, their soluble proteins were extracted and tested with and without supplementation. The present communication describes the extraction, isolation and purification of proteins from these wild leguminous seeds and their evaluation by animal feeding experiments.

Incidentally, as liver tissue and liver enzymes are the most sensitive to respond to alterations in dietary protein, both qualitatively and quantitatively, liver

protein depletion and repletion studies and assays of some important liver enzyme systems that respond significantly during altered conditions of protein feeding, were carried out.

### MATERIALS AND METHODS

Purified protein isolates were prepared as described earlier<sup>2</sup>.

*Evaluation of Proteins by Animal Experiments.*—The biological values of the isolated proteins and their protein efficiency ratios were determined by animal experiments on 12 albino rats per group, 4–5 weeks old and weighing 40–48 g. Both the balance sheet method<sup>3,4</sup> and the rat growth method<sup>5,6</sup> were employed.

*Experimental Diets.*—A diet practically nitrogen-free but adequate in all other respects was prepared. It contained soluble starch (analytical reagent grade) 80 parts; sucrose, 10 parts; groundnut oil, 6 parts;



salt mixture<sup>7</sup>, 2 parts; vitamin mixture, 1 part; and cellulose powder, 1 part. In experimental diets starch was replaced by dietary protein at 10% level.

Vitamin mixture had the following composition per tablet in mg: Inositol, 2.2; Choline chloride, 75; Niacin, 2.0; Riboflavin, 0.4; Pyridoxine-HCl, 0.2; Thiamine-HCl, 0.4; Calcium pantothenate, 1.2; Folic acid, 40 and vitamin B<sub>12</sub> 600 µg. One tablet of vitamin mixture was employed per 100 g of the diet. Shark liver oil 5 drops per rat twice a week was administered.

All diets were cooked and fed to animals as detailed earlier<sup>2</sup>.

The liver protein repletion method of Harrison and Long<sup>8</sup> was employed for determining the liver repletion capacity of the wild leguminous seed protein isolates.

Albino rats weighing between 100–160 g were standardized for one week on a diet containing 20% casein. After one week, one group of six animals was sacrificed and total liver protein<sup>9</sup>, albumin<sup>10</sup>, glycogen<sup>11,12</sup>, and activities of some enzyme systems like active phosphorylase<sup>13</sup>, succinic dehydrogenase<sup>14</sup>, catalase<sup>15</sup>, alkaline and acid phosphatase<sup>16,17</sup> and xanthine oxidase<sup>18</sup> were assayed. Subsequently, after fasting all the animals for 48 hours one more group of six animals was sacrificed and all the aforesaid liver assays were repeated. One of the remaining groups of animals

was then fed on a 10% casein diet while all the others were maintained on the experimental protein diets at 20% level both in the unsupplemented form as well as supplemented with 0.2% of L-tryptophan and 0.15% of L-methionine. At the end of the experimental period of four days, all the animals (6 in each group) were sacrificed and the liver assayed for total proteins and the various enzyme systems.

#### RESULTS AND DISCUSSION

Most of the experimental unsupplemented protein isolates when fed to the experimental animals failed to promote growth. However, when supplemented with the limiting amino acids methionine and tryptophan, recorded protein efficiency ratios (PER) between 1.0–1.7 and net protein ratios (NPR) between 1.9–2.0 (Table I) as against 2.55 and 3.27 respectively for casein at 10% level. The PER figures are much lower than for casein and also induce less growth (12.6–25.5 g) in comparison with casein (39.8 g) during the same period. The Biological Values (B.V.) and the Digestibility Coefficients (D.C.) range respectively between 43–63 and 72–84 and are not anywhere near for those of casein. This suggests that the wild seed protein isolates although nutritionally much inferior to casein, on supplementing with the limiting amino acids are capable of promoting growth and also maintain nitrogen balance in experimental animals.

TABLE I

Protein efficiency ratio and net protein ratio of some uncultivated leguminous seed protein isolates

Protein source	Weight change rat in 4 weeks (g)	PER	NPR	B.V.	D.C.
Nitrogen free (12)	—13.5				
Casein (10)	39.8 ± 1.7	2.55	3.27	92.0	96.0
<i>Acacia arabica</i> (12)	—3.8				
* <i>Acacia arabica</i> (9)	25.5 ± 4.7	1.72	2.47	52.0	83.0
<i>Acacia catechu</i> (10)	—6.1				
* <i>Acacia catechu</i> (11)	17.6 ± 1.7	1.47	2.42	46.0	84.0
<i>Albizia moluccana</i> (12)	—7.9				
* <i>Albizia moluccana</i> (11)	20.2 ± 3.5	1.45	2.25	43.0	83.0
<i>Albizia richardiana</i> (10)	21.2 ± 2.5	1.52	2.30	61.6	78.6
<i>Bauhinia macrostachya</i> (8)	—9.5				
* <i>Bauhinia macrostachya</i> (8)	14.2 ± 1.6	1.05	2.05	63.0	82.0
<i>Bauhinia malabarica</i> (18)	—5.3				
* <i>Bauhinia malabarica</i> (12)	12.6 ± 2.7	1.38	2.85	55.7	75.3
<i>Bauhinia variegata</i> (11)	—8.1				
* <i>Bauhinia variegata</i> (9)	16.1 ± 1.8	1.05	1.93	56.6	72.2
<i>Cassia absus</i> (8)	—4.9				
* <i>Cassia absus</i> (9)	13.5 ± 2.6	1.00	2.00	47.9	75.8
<i>Leucira glauca</i> (10)	13.3 ± 3.8	1.16	2.10	37.0	78.0
<i>Pithecellobium dulce</i> (11)	—8.1				
* <i>Pithecellobium dulce</i> (12)	24.8 ± 2.0	1.54	2.37	62.6	77.5

Figures in brackets denote the number of animals.

\* Supplemented with L-Methionine (0.15%) and L-Tryptophan (0.20%). Dietary protein was at 10% level and the rats employed initially weighed between 40–48 g.

Table II shows that rats fed on 20% casein diet for one week had about 21% liver protein. After 48 hours of fasting it declined to 15% which on resumption of the casein diet re-attained almost the original level. On feeding the fasted animals with the unsupplemented diets, the liver protein levels further declined. However, feeding of diets, supplemented with methionine and tryptophan, brought about some improvement in liver protein.

Increase of glycogen in the liver tissue in some cases and depletion thereof in others, have been observed during feeding of unsupplemented test diets after fasting period. This could be attributed to the derangement caused in the normal carbohydrate metabolism, probably by the unbalanced amino acid pattern of the diets resulting in the failure to resynthesize the enzymes required for glycogen synthesis and breakdown. This assumption

TABLE II

Repletion studies on liver proteins and glycogen in rats during feeding of protein isolates from some uncultivated leguminous seeds

Diet	Liver wt. (g)	% Liver protein	% Liver albumin	% Liver glycogen (mg %)
20% Casein	4.99±0.61	21.0±3.0	6.6±1.6	344±54
Fasting	3.23±0.53	15.1±1.3	4.8±0.7	84±23
10% Casein	4.75±0.48	19.9±1.3	6.4±1.3	366±83
<i>Acacia arabica</i>	4.31±0.59	16.2±2.3	5.7±2.2	96±29
* <i>Acacia arabica</i>	5.03±0.76	17.9±3.6	6.0±2.8	223±38
<i>Acacia catechu</i>	4.49±0.67	15.1±2.5	5.6±1.2	100±32
* <i>Acacia catechu</i>	4.63±0.63	17.0±3.4	6.3±0.9	289±39
<i>Acacia melanoxylon</i>	5.05±0.81	16.5±2.0	4.9±1.7	109±19
* <i>Acacia melanoxylon</i>	4.38±0.59	17.3±2.2	6.2±2.1	242±23
<i>Albizia richardiana</i>	6.35±1.10	17.0±4.3	5.3±1.9	205±28
<i>Bauhinia macrostachya</i>	5.86±1.16	13.7±3.1	5.1±1.6	80±28
* <i>Bauhinia macrostachya</i>	4.49±0.85	14.5±3.4	6.2±2.3	169±41
<i>Bauhinia malabarica</i>	4.86±1.02	13.5±2.6	4.9±1.3	95±21
* <i>Bauhinia malabarica</i>	3.96±1.01	14.2±2.9	5.8±2.0	179±26
<i>Bauhinia variegata</i>	5.22±0.39	13.8±2.5	3.3±0.4	79±49
* <i>Bauhinia variegata</i>	4.51±1.48	14.3±0.9	5.4±2.5	189±19
<i>Cassia absus</i>	4.42±0.89	13.6±1.3	4.4±1.1	104±18
* <i>Cassia absus</i>	5.55±0.98	16.4±1.8	5.2±1.5	153±15
<i>Pithecellobium dulce</i>	4.14±0.43	12.3±1.2	5.0±2.1	93±24
* <i>Pithecellobium dulce</i>	4.87±0.41	15.6±2.1	5.4±1.2	203±37

\* Supplemented with L-Methionine (0.15%) and L-Tryptophan (0.20%).

Studies on liver catalase and alkaline phosphatase (Table III) showed that during starvation the activity significantly increased which on feeding of unsupplemented diets did not improve. However, the supplemented protein diets brought down the activity. Acid phosphatase, succinic dehydrogenase and xanthine oxidase activity during starvation and on unsupplemented incomplete protein diets depicted marked decline which, however, got enhanced on feeding the animals with supplemented diets (Table III).

The enhanced active phosphorylase activity accompanied with liver glycogen depletion could be interpreted in the light of increased metabolic reactions with enhanced rate of glycogen breakdown in the liver tissue. This could also be due to the hormonal stimulation caused by the strain during starvation. However, the overall improvement in all the enzymic activities as well as in glycogen concentration points out and emphasizes the nutritional adequacy of the supplemented test proteins.

tion is partly lent support by an earlier report<sup>19</sup> which points out that accumulation of liver glycogen is characteristic during protein deficiency.

Based on the above investigations, it could be concluded that the wild leguminous seed protein isolates have a fairly balanced amino acid pattern although in most of them methionine and tryptophan happen to be the limiting factors. However, when fortified with the missing essential amino acids, the experimental animals consumed the diets with as much avidity as for any other normal diet and what is more no untoward symptoms whatsoever were noticed in them.

The supplemented protein isolates also proved capable of maintaining positive nitrogen balance and restored normal growth in experimental animals. The results while compared with those obtained for some edible seeds<sup>20</sup> with respect to PER, weight gain, Biological Value and Digestibility Coefficient, etc., indicate the supplemented uncultivated seed protein isolates to be nutritionally at par—if not

TABLE III

Studies on some rat liver enzymes during feeding of protein isolates from some uncultivated leguminous seeds

Diet	Alkaline phosphatase <sup>a</sup>	Acid phosphatase <sup>b</sup>	Active phosphorylase <sup>c</sup>	Catalase <sup>d</sup>	Xanthine oxidase <sup>e</sup>	Succinic dehydrogenase
20% Casein	2.75±1.41	5.31±2.35	3.78±1.91	17.24±1.83	275.0±17.0	2802±86
Fasting	3.5±2.41	1.99±1.01	6.03±1.65	24.96±1.25	78.7±11.2	956±28
10% Casein	2.16±0.97	4.28±0.61	4.24±0.85	16.53±2.12	176.9±11.2	2132±71
<i>Acacia arabica</i>	5.40±2.75	2.91±0.23	5.63±1.32	19.92±2.25	89.5±10.9	1505±13
* <i>Acacia arabica</i>	4.62±2.32	4.87±0.65	3.93±0.69	18.11±1.63	132.6±15.4	2430±86
<i>Acacia catechu</i>	5.65±2.72	2.88±0.16	4.98±0.86	20.76±1.06	73.9±13.6	1480±18
* <i>Acacia catechu</i>	4.91±1.62	5.49±1.36	3.71±1.13	19.01±1.78	152.4±16.2	2005±13
<i>Acacia melanoxylon</i>	4.32±1.16	2.32±2.13	5.95±1.32	19.98±2.01	115.0±14.6	1932±15
* <i>Acacia melanoxylon</i>	4.00±1.93	4.45±1.64	4.08±0.93	18.26±1.59	154.0±18.9	2020±21
<i>Albizia richardiana</i>	4.11±1.26	2.93±1.83	4.51±0.75	16.66±6.20	129.0±17.1	1978±14
<i>Bauhinia macrostachya</i>	6.76±6.02	3.16±0.36	5.23±1.25	19.49±3.37	69.8±15.6	2220±25
* <i>Bauhinia macrostachya</i>	5.64±3.14	5.30±0.70	4.22±0.78	18.62±1.86	148.6±12.9	2495±32
<i>Bauhinia malabarica</i>	6.85±2.14	4.30±1.18	5.99±1.42	22.13±3.82	65.9±10.6	2380±29
* <i>Bauhinia malabarica</i>	5.12±1.42	4.60±0.91	4.62±0.89	18.44±3.08	152.3±12.1	2415±53
<i>Bauhinia variegata</i>	5.47±1.72	1.02±0.12	4.50±0.73	19.56±2.19	79.3±14.1	1839±46
* <i>Bauhinia variegata</i>	4.45±1.35	3.41±1.27	3.81±1.24	16.83±1.96	161.1±12.3	2125±86
<i>Cassia absus</i>	7.98±0.93	3.90±1.13	6.10±0.92	19.49±7.15	109.0±9.3	1895±17
* <i>Cassia absus</i>	4.92±0.74	5.20±2.17	4.30±1.00	16.33±4.77	180.0±13.6	2243±21
<i>Pithecellobium dulce</i>	4.96±0.59	1.36±0.11	5.26±0.94	21.95±3.18	98.3±11.1	1070±89
* <i>Pithecellobium dulce</i>	3.95±1.07	3.50±0.64	3.50±0.56	18.59±2.06	169.0±12.7	1517±53

\* Supplemented with L-Methionine (0.15%) and L-Tryptophan (0.20%).

<sup>a</sup>, <sup>b</sup> mg P liberated/g liver protein/hour, <sup>c</sup>  $\mu$ g P liberated/mg liver protein/30 min., <sup>d</sup> Residual O<sub>2</sub> in mg after 10 min. catalase activity at 2° C/g liver protein, <sup>e</sup>  $\mu$ l O<sub>2</sub> consumed/g wet liver/hour, <sup>f</sup>  $\mu$ g formazan formed/mg wet liver.

better—with the edible seeds. Furthermore, they do not appear to induce any deleterious physiological after-effects on the experimental animals, as evinced by liver protein and several liver enzyme assays.

#### ACKNOWLEDGEMENT

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# ORIGIN, NATURE AND LIMIT OF POLYPLOIDY IN MARIGOLDS

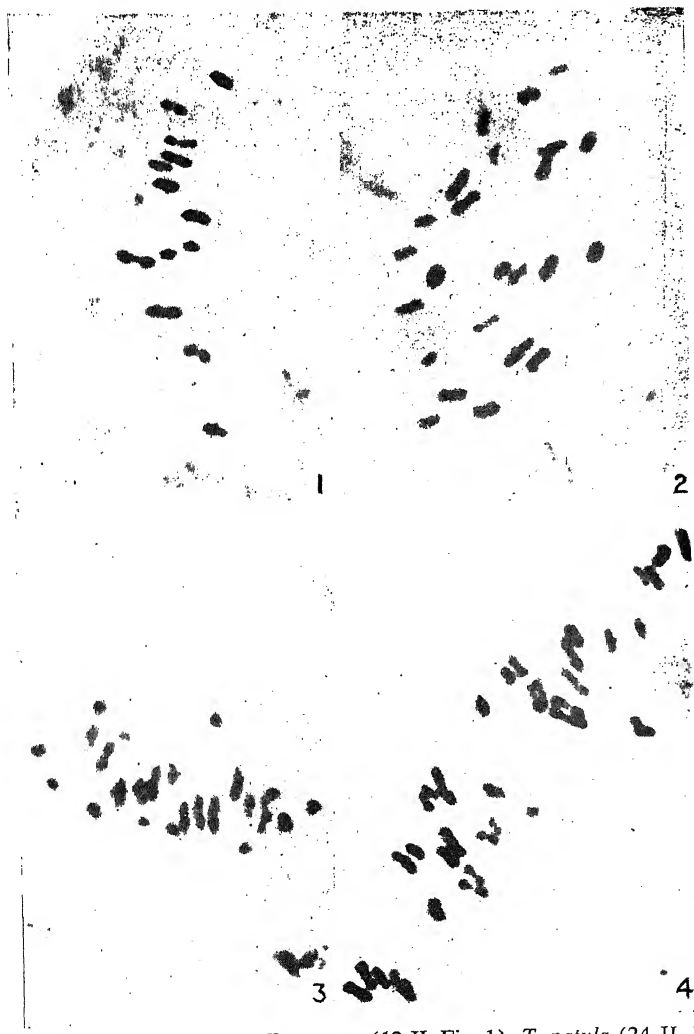
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THE two marigolds, the African (*Tagetes erecta* Linn.) and the French (*T. patula* Linn.), are actually natives of Mexican region and their popular names are misleading inasmuch as these have been acquired during their roundabout entry in Europe. In India these were introduced by Portuguese<sup>7</sup> and spread quickly because of the ease in cultivation, adaptability to varying soil and climatic conditions, longer blooming period and beautiful flowers that have excellent keeping quality. At present, marigolds constitute one of the five most commonly cultivated

and used flowers in urban and rural India. Furthermore, the rapid diffusion of marigolds both in India and Southern Europe by the Portuguese and Spanish indicates that, during the pre-Columbian domestication, these species had become sufficiently attractive ornamentals in the region of their origin and there is ethno-botanical evidence for their long selection history in relation to religious ceremonies which led to morphological diversification in them<sup>3</sup>.

*T. erecta*, a diploid ( $n = 12$ ; Fig. 1), is generally tall (about 90 cm) with large double flowers.



FIGS. 1-4. Metaphase I in pmc in *T. erecta* (12 II, Fig. 1), *T. patula* (24 II, Fig. 2), F<sub>1</sub> *T. erecta* × *patula* (12 II + 12 I, Fig. 3) and amphiploid *T. erecta-patula* (2 IV + 32 II, Fig. 4), × 1,500.

Heterosis has been exploited in this species with remarkable success and  $F_1$  hybrids are medium tall with often very large (15.2 cm across) uniform flowers. Even types with odourless foliage have been evolved. *T. patula*, on the other hand, is a dwarf (15–45 cm) tetraploid ( $n=24$ ; Fig. 2) species with relatively small flowers (2.5–5.1 cm). While former has orange to very light lemon coloured flowers, in the latter the colour varies from golden yellow to rusty red and all combinations in between.

The tall habit and smell of the leaves of *T. erecta* have been generally regarded as undesirable characters. For this reason, triploid interspecific hybrids (*T. erecta*  $\times$  *patula*) have been marketed in the USA and have been preferred because they combine the characters like relatively large double flowers (5.1–7.6 cm) with medium tall to dwarf habit, and diversity in colour and prolificity of flowering. On an average the triploids show 0.1 III + 12.35 II + 11.0 I (Table I) at metaphase I in male meiosis. The most common association being 12 II + 12 I (Fig. 3). Meiosis is highly irregular leading to sterility which confers the character of continued blooming.

there is morphological diversity and also variation in fertility. There is, however, no correlation between chromosome number, morphology and fertility of  $C_1$  segregates.

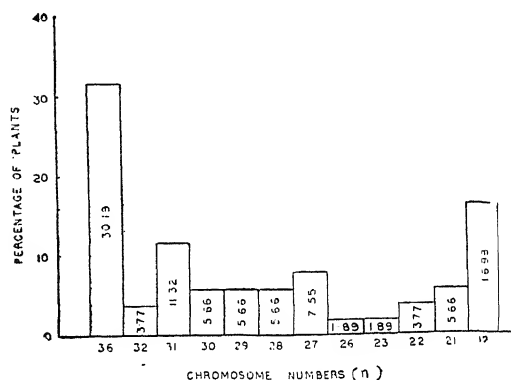


FIG. 5. Chromosomal variation in  $C_1$  progeny of amphiploid *T. erecta-patula*.

Based on the morphological and cytogenetical evidence, Towner<sup>6</sup> and Bolz<sup>1</sup> have concluded that *T. patula* ( $2n=48$  ApAp BpBp) is probably of amphidiploid origin between *T. erecta* ( $A_1A_1$ ) and

TABLE I  
Chromosome associations, chiasma frequency and fertility in species and hybrids of marigold  
(Range is given within brackets)

Taxon	2n	Associations at metaphase				Xa frequency	Fertility (%)	
		IV	III	II	I		Pollen	Seed
<i>T. erecta</i>	24	..	..	11.9 ± 0.02 (11–12)	0.2 ± 0.04 (0–2)	20.25 ± 0.59 (17–22)	76.46	91
<i>T. patula</i>	48	..	..	24	..	42.65 ± 0.605 (38–46)	92.14	94
<i>T. erecta-patula</i>	36	..	0.1 ± 0.02 (0–1)	12.35 ± 0.007 (9–16)	11.0 ± 0 (4–18)	23.4 ± 1.08 (15–30)	0–33	0
<i>T. erecta-patula</i>	72	0.75 ± 0.012 (0–2)	..	33.95 ± 0.04 (30–36)	1.1 ± 0.09 (0–6)	59.65 ± 1.70 (45–69)	57.28	42

Colchi-hexaploids (Fig. 4) from the triploid hybrid showed generally 36 II but on an average there are 0.75 IV + 33.95 II + 1.1 I with reasonable male and female fertility (Table I). The  $C_1$  progeny of the amphiploid *T. erecta-patula* was morphologically and cytologically very heterogeneous (Fig. 5). Out of the 80 plants raised, 68 were analysed in detail and, of these, only 30.18% had the parental  $6x$  number, while in the remaining 69.82% the number varied from  $2n=64$  ( $6x-8$ ) to 24 ( $2x$ ). The plants with diploid number constituted nearly 17% being next highest to the  $6x$  level. Parallel to cytological heterogeneity,

*T. tenuifolia* ( $B_1B_1$ ) (both  $2n=24$ ), or of taxa closely related to them. The two species show a strong reproductive barrier leading to very few good seeds in  $F_1$  hybrid, seedling mortality, hybrid weakness and sterility. There are on an average hardly 4.4 II (range 0–11 II). On the other hand, the colchicine amphidiploids correspond morphologically with *T. patula* and, like it, are fertile with 24 II as a result of perfect preferential pairing. The extent and nature of homology between A genomes of *T. erecta* and *T. patula* can be assessed from the meiotic behaviour and fertility of the hybrid *T. erecta*  $\times$  *patula* and its amphiploid *T. erecta-*

*patula*. The *Drosera* scheme (12 II + 12 I) and total sterility in the former, is not consistent with 36 II and reasonable fertility in the latter. Thus A genomes in *T. erecta* and *T. patula* do not appear to correspond exactly as is also clear from the total sterility in  $F_1$  triploid hybrid. Obviously, the genomic formulae are only approximations and do not indicate homology/non-homology in absolute terms<sup>4</sup>. It cannot be said with certainty if the divergence in the prototype *T. patula* took place subsequent to its origin or that some closely related species to *T. erecta* has been the source of its A genome. Thus, out of the three genomes of hexaploid ( $A_1A_1 ApAp BpBp$ ), two, though related, are, however, not able to work harmoniously, and this amphiploid is a typical segmental allohexaploid in character<sup>5</sup> in that it possesses, apart from bivalents, a low multivalent frequency, partial fertility (Table I) and segregates genetically for parental characters due to inter-genomal pairing. Obviously, such a condition is not stable and must segregate in the direction of auto- or allo- or stable segmental allopolyploidy. Thus, it must undergo a period of rigorous selection for fertility and stability of desired morphological attributes. This is possible only when heterogenetic associations get restricted due to loss of large duplicated loci. The recovery of about 17%

diploids from the progeny of amphiploid *T. erecta-patula* is not an indication of the phenomenon of depolyploidy<sup>2</sup> as it is not a case of enbloc segregation of  $A_1$  or Ap genomes, because the diploid progeny does not resemble diploid parental species in morphology or fertility.

The present results tend to indicate that hexaploidy may not be successful in marigolds. The highest level of ploidy reported in about 50% of the species<sup>6</sup> of the genus *Tagetes* is tetraploidy. Furthermore, the long course of domestication has not been able to establish hexaploidy in *T. erecta* and *T. patula* complex, although there must have been ample opportunities for the same during the course of domestication extending for over 400 years.

Our thanks are due to Mr. A. K. Sengupta and Mr. T. K. Sharma for illustrations.

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## SEROLOGICAL GROUPING OF THE INDIAN BACTERIOPHAGES OF *XANTHOMONAS ORYZAE* (UYEDA AND ISHIYAMA) DOWSON

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THE importance of serological relationships in characterization and classification of bacteriophages including those affecting *Xanthomonas oryzae* has been emphasized by earlier workers<sup>1-5</sup>. In India, Rao<sup>6</sup> isolated phages for seventy-two isolates of *X. oryzae* collected from various parts of the country and grouped them into fourteen distinct strains based only on their physical properties and host range. The present work, therefore, was undertaken to study the serological behaviour of these phages.

### MATERIALS

Two phages, viz.,  $XOP_{14}$ <sup>6</sup>, polyphagous bacteriophage of Indian origin and  $OP_2$ , a well-studied

phage established by Japanese workers were used. Both these phages were grown on  $S_4$  strain of *X. oryzae* (Cultures of the bacterium and phages were obtained from Dr. Y. P. Rao, I.A.R.I., New Delhi).

### PREPARATION OF HIGH TITRED $XOP_{14}$ AND $OP_2$ PHAGE STOCKS

*X. oryzae* strain 4 was allowed to grow in P.G.S. broth having Peptone (10 g), Glutamic acid (0.5 g) and Sucrose (10 g) under aerobic conditions in a water bath (27°–30° C) provided with reciprocal shaker arrangements. When the population reached  $5 \times 10^7$  cells/ml, approximately 500 phage particles each of  $XOP_{14}$  and  $OP_2$  were added separately to each ml of the bacterial culture. The number of

plaque forming particles/ml was assayed by the double layer technique<sup>7</sup>. The turbidity of the bacterial culture was found to decline gradually reaching the maximum after 18 hr of incubation. The lysed culture (lysate) was centrifuged once at 1,500 rpm for 15 minutes and the supernatant was collected and kept at 40° C for a week. This was centrifuged again at 500 rpm for 5 minutes and the supernatant was stored in a deep freeze after checking the number of plaque forming units/ml. The antigens thus prepared were used for the preparation of antiserum.

#### PREPARATION OF ANTISERA

Three rabbits (1.3 kg each) were inoculated by each of the two phages (XOP<sub>14</sub> and OP<sub>2</sub>) intravenously in ear veins at the rate of 2.5 ml of phage (10<sup>10</sup> plaque forming units/ml). Seven injections were given on alternate days. Four days after the last injection, five ml of blood were collected from ear-marginal vein and heart of each rabbit. The blood samples, in petrolatum-lined centrifuge tubes, were left to clot at 37° C and then in a refrigerator overnight. The remaining fluid was centrifuged at 500 rpm for 5 minutes, the supernatant was held at 56° C for 10 minutes to remove non-specific inhibitors. This was then filtered through sterile sintered glass filters. The antiserum thus obtained was stored in screwcap vials at 4° C for further studies.

#### SPOT NEUTRALIZATION TEST

Employing the antisera of XOP<sub>14</sub> and OP<sub>2</sub>, the serological relationship between these and five others, viz., P<sub>3</sub>, P<sub>5</sub>, P<sub>6</sub>, P<sub>11</sub> and P<sub>12</sub> (having narrow and wide host range) were tested. A mixture of 0.1 ml of phage suspension (250–300 particles/ml) and 1.0 ml of the host-bacterial suspension was added to 5 ml of molten PGSA medium (containing 1.5% agar) and poured over a primary layer of 2.0% plain agar in petriplates. After the top layer solidified, drops of specific antisera were placed, by means of a pipette, on previously marked places. In homologous systems, plaques failed to appear in the areas covered by the antiserum and instead, the bacterial growth occurred. The results are given in Table I.

From the results, it is apparent that all the six Indian phages tested were found to be serologically related to the OP<sub>2</sub> phage which is of Japanese origin. It is interesting to note that all the Indian phages, tested here, included strains with wide as well as narrow host-range. Yet it is difficult to exclude the possibility, of serotypes of OP<sub>1</sub>, OP<sub>1</sub>h and OP<sub>1</sub>h<sub>2</sub> or any other hitherto unidentified phage of *X. oryzae* not occurring in India unless a large number of Indian isolates are examined.

TABLE I  
Serological relationship amongst strains of phages of *Xanthomonas oryzae*

Phage strains	Reactions		
	Normal/serum	OP <sub>2</sub> antiserum	XOP <sub>14</sub> antiserum
*XOP <sub>3</sub>	—	+	+
XOP <sub>6</sub>	—	+	+
XOP <sub>10</sub>	—	+	+
XOP <sub>11</sub>	—	+	+
XOP <sub>12</sub>	—	+	+
XOP <sub>14</sub>	—	+	+
OP <sub>2</sub>	—	+	+

+ Plaques failed to appear indicating +ve neutralization, — Plaques appeared indicating —ve reaction.  
\*XOP<sub>3</sub> was grown on S<sub>3</sub> strain of *X. oryzae* while the others were grown on S<sub>4</sub> strains in which both XOP<sub>14</sub> and OP<sub>2</sub> phages produced smaller and solid plaques.

Another important information available from this study is that serological reactions of the phages are not related to their host-range. Between XOP<sub>3</sub> and XOP<sub>14</sub> which are serologically alike, the former was specific to a single strains of *X. oryzae* whereas the latter had a very wide host-range, typing all the ten strains of the bacterium and even other species of *Xanthomonas*. Thus, serological typing of phages of *X. oryzae* alone would offer little prospect of tracing the source, origin and movement of the Indian strains of the bacterium from one rice locality to another or identify the distributional pattern of the bacterial disease in an epidemic year within the country. Lack of correlation between serological reactions and host-range specificity among wild type related even phages of *Escherichia coli* has been reported by Yadava, Chandra and Gupta<sup>8</sup>.

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## LETTERS TO THE EDITOR

### KINETIC AND SOLVENT ISOTOPE EFFECTS IN OXIDATION OF ACETOPHENONES BY VANADIUM(V)

LITTLER AND WATERS<sup>1</sup> and Littler<sup>2</sup> have concluded that oxidation of cyclohexanone and cyclopentanone by vanadium(V) under acid conditions involves a direct attack on ketone. The conclusion was based on the criterion developed by Best, Littler and Waters<sup>3</sup>. The same conclusions have been derived by Bhargava<sup>4</sup> in oxidation of some acyclic ketones by vanadium (V). A primary kinetic isotope effect,  $k_H/k_D$ , ( $= 4.2$ )<sup>1</sup> in the oxidation of cyclohexanone by V(V) indicated a C-H bond rupture in the transition state. Present communication reports the results of a study of kinetic and solvent isotope effects in oxidation of acetophenone ( $\alpha$ -D) by vanadium(V) under acid conditions.

The reaction was studied spectrophotometrically by following the rate of formation of vanadium (IV) species at 680 and 750 nm. Reacting solutions were thermostated in glass stoppered bottles before mixing and transferring it to the cell tube. The cell tube was surrounded by a metallic block through which water was circulated from thermostat ( $\pm 0.02^\circ\text{C}$ ). The error in the measurement of rate constants is  $\pm 5\%$ .

All the reagents used were either chemically pure or were purified using conventional techniques. Deuterated acetophenone ( $\alpha$ -D) was prepared by the method of Jones *et al.*<sup>5</sup> and was found by PMR to contain 33% of D content. For solvent isotope effect heavy water (99.8% D content) supplied by BARC, Bombay, was used as such. Vanadium (V) solution was prepared in  $\text{D}_2\text{O}$  using  $\text{NH}_4\text{VO}_3$  and conc.  $\text{H}_2\text{SO}_4$ .

Table I summarises the data on the solvent isotope effect. Using the criterion of Best, Littler and Waters a value of  $k_{\text{D}_2\text{O}}/k_{\text{H}_2\text{O}} = 1.7$  approaches the one predicted for the attack on the ketone form. An apparent primary kinetic isotope effect,  $k_H/k_D = 1.76$  can be evaluated from the data of

TABLE I

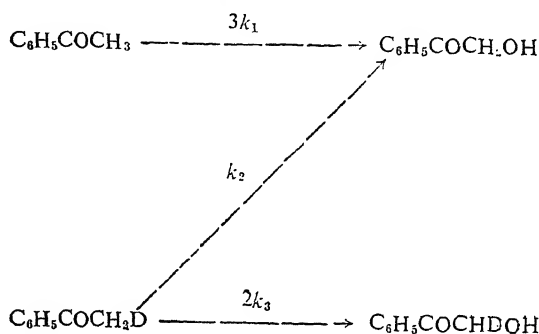
Kinetic and solvent isotope effects

[Ketone]: 0.83 M;  $[\text{H}_2\text{SO}_4]$ : 0.8 M; Temp.:  $50^\circ$ ;  
Solvent: 60% AcOH (v/v)

Substrate	$k_1 \times 10^5 (\text{sec}^{-1})$	$k_1 \times 10^5 (\text{sec}^{-1})^\dagger$
$\text{C}_6\text{H}_5\text{COCH}_3$	9.36	15.8
$\text{C}_6\text{H}_5\text{COCH}_2\text{D}$	5.30	9.1

\* Mean of triplicate runs,  $^\dagger$  In 33% (v/v)  $\text{D}_2\text{O}$

the table (column 1). Since the sample is only 33% deuterated, the following paths can be written for its oxidation by vanadium (V) assuming the rate limiting step to involve a direct



attack on ketone to produce the intermediate hydroxy ketone. Using the statistical corrections, the pseudo first order rate constant for the oxidation of the protiated compound,  $k_1$ , is  $3.12 \times 10^{-5} \text{ sec}^{-1}$ . The rate of oxidation of the deuterated analogue would then be  $k_2 + 2k_3$ , where  $k_3$  stands for the attack on the protons and  $k_2$  that on the deuterons of  $\text{C}_6\text{H}_5\text{COCH}_2\text{D}$ . In the oxidation of cyclohexanone by V(V), a primary kinetic isotope effect,  $k_H/k_D = 4.2$  has been observed by Littler and Waters<sup>1</sup> showing the tendency to approach a theoretical value of 5.0. Assuming, in the present case, a value of  $k_H/k_D (= k_1/k_2) = 5.0$  one can calculate  $k_1/k_3$ , the secondary isotope effect = 1.46 from the following relation:

$$3k_1/(k_2 + 2k_3) = 9.36/5.30 = 1.76.$$

Secondary isotope effect of this magnitude has been observed<sup>6</sup>. Thus the observed value of kinetic isotope effect can be explained by assuming a primary kinetic isotope effect of 5.0 and a secondary isotope effect of 1.46.

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### EFFECT OF SALINITY ON RNA AND PROTEIN BIOSYNTHESIS IN WHEAT SEEDLINGS

PLANTS grown in saline soil, in general, are characteristically small in size and stunted in growth, although their appearances are normal. Increased osmotic pressure in the root zone due to salinity may not be the only factor responsible for the retardation in growth. It is more than a simple moisture stress phenomenon<sup>1</sup>. Studies on the physiological processes like photosynthesis<sup>2</sup>, respiration<sup>2</sup> and the activities of certain enzymes<sup>3</sup> as influenced by saline conditions have not elucidated the mechanism. It is known that the ionic strength in the cells influences the ionic atmosphere around the nucleic acid phosphate backbone<sup>4,5</sup>, which in turn may affect  $t_m$  and the stability of DNA<sup>7,8</sup> and the interaction between the nucleic acid and protein<sup>9</sup>. As these components are involved in the biosynthesis of nucleic acids and proteins, the effect of salt concentrations on these biosyntheses was studied. Since the seedling stage has a vigorous biosynthetic activity, wheat seedlings were used in the present study.

Seedlings of wheat variety S 227 were raised in sand culture containing the nutrient medium described by Hewitt<sup>10</sup>. Ten days old seedlings were selected for the study. Incorporation of radioactive uracil and radioactive leucine into RNA and protein was used to measure the biosyntheses of RNA and protein, respectively. Incubation medium for the measurement of RNA biosynthesis was 0.01 M phosphate buffer, pH 6.8, containing 0.1 mM  $\text{CaSO}_4$ , 2  $\mu\text{Ci}$  uracil-2- $\text{C}^{14}$  (specific activity 46.7 mCi/m mole) per tube and different NaCl concentrations (0, 40, 80, 120 meq/l) and for measurement of protein synthesis, it was 0.01 M phosphate buffer, pH 5.2, containing glucose 0.1%, 2  $\mu\text{Ci}$  leucine-1- $\text{C}^{14}$  per tube (specific activity 41.5 mCi/m mole)<sup>12-14</sup> and different salt concentrations used. Further treatment was same in both the cases. The reaction was stopped by transferring the tissues to 75% alcohol and boiling for two minutes. Tissues were pulverised and washed five times with 75% alcohol containing 0.2  $\mu$  mole/ml of uracil or leucine (both cold). The residue was suspended in 5 ml of 0.3 N sodium hydroxide for 18 hours at 30° C followed by centrifugation. The

residue was once again suspended in 5 ml of 0.3 N sodium hydroxide, kept for two hours at 30° C and then centrifuged. Both the supernatants were combined, concentrated and transferred to planchets for drying and measurement of radioactivity (G.M. Counter, Electronic Corporation of India, Hyderabad). Uracil-2- $\text{C}^{14}$  and leucine-1- $\text{C}^{14}$  were obtained from Isotope Division, Bhabha Atomic Research Centre, Trombay, Bombay.

Figure 1 shows the effect of salt on the incorporation of labelled uracil into RNA of wheat seedlings.

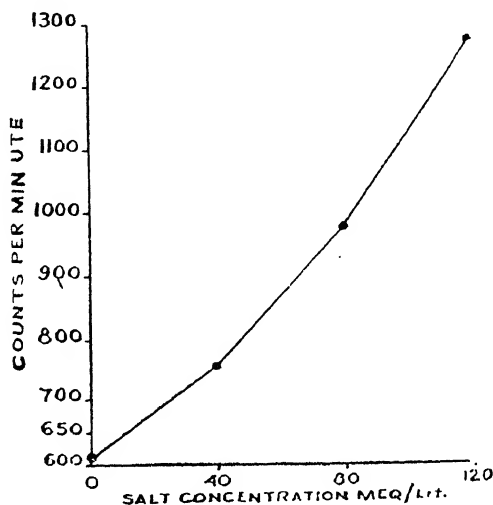


FIG. 1. Effect of salinity on incorporation of uracil-2- $\text{C}^{14}$  into RNA fraction of wheat seedlings.

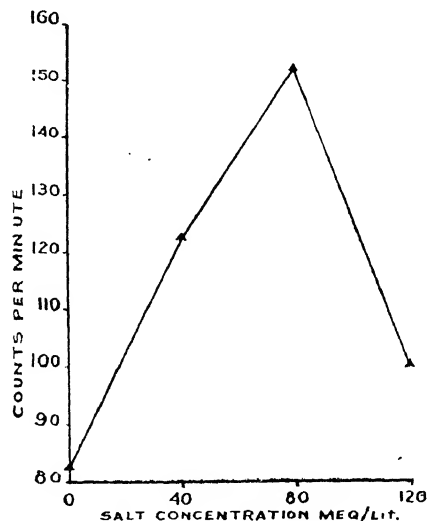


FIG. 2. Effect of salinity on incorporation of leucine 1- $\text{C}^{14}$  into protein fraction of wheat seedlings.

The data indicate that the presence of salt stimulates the RNA biosynthesis as demon-

strated by the increase in the incorporation of uracil-2-C<sup>14</sup> into alcohol insoluble RNA fraction. The increase was linear with the corresponding increase in salt concentration in the incubation medium. The results summarised in Fig. 2 show leucine-1-C<sup>14</sup> incorporation into the protein fraction of wheat seedlings. Protein biosynthesis increased upto a salt concentration of 80 meq/l followed by a decrease at 120 meq/l. Comparing the data in the two figures it becomes apparent that there was parallel increase in the biosyntheses of RNA and protein upto a NaCl concentration of 80 meq/l. After this the RNA synthesis kept on increasing, but the protein synthesis dropped rapidly. During protein synthesis, the message contained in messenger RNA is translated into protein with the help of ribosomes, transfer RNA, amino acyl t-RNA ligases and other protein synthesising factors. Therefore, the concomitant increase in protein synthesis may be the result of an increase in RNA synthesis. It is difficult at this time to explain the decrease in protein synthesis at the salt concentration of 120 meq/l, while the RNA synthesis was still progressing. It may perhaps be due to the influence of salt on certain processes during translation.

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## TECTONICS OF THE REGION OF EASTERN HIMALAYAN SYNTAXIS

To study the tectonic history of the Eastern Himalayan Syntaxis, the authors have undertaken traverse mapping in parts of Subansiri, Siang and Lohit districts of Arunachal Pradesh, NEFA, Himalaya.

Four litho-tectonic units, showing ENE-WSW regional trend, have been recognised in the Siang district<sup>1</sup>. The Upper Tertiary sediments of Siwalik Group are overridden with a thrust contact by Gondwana formation (Upper Palaeozoic<sup>2,3</sup>) which in turn are thrust over by sedimentary and volcanic members of the Miri Group and metamorphites of the Siang Group. In the Tuting area, lower Tsangpo valley, a thrust sheet of gneissose granite body overlies the Siang Group of metamorphites. Analysis of structural elements, such as orientations of folds and thrusts, in these litho-tectonic units indicate a major compressional phase of tectonic movement of Himalayan age that was directed in NW-SE direction. This tectonic episode has affected rocks as young as the Upper Tertiary Siwalik Group.

In the adjoining Lohit district to the east of Siang district, three major litho-tectonic units, trending NW-SE and dipping northeastward, have been postulated by the authors and Nandy<sup>4</sup>. The Mishmi Metamorphites, whose base delineated by a thrust, i.e., Mishmi Thrust, is overlain by the Tiding Ophiolite Zone which in turn is thrust over by the Lohit Granodiorite Diorite body.

The rocks of Gondwana and Siwalik belts, trending ENE-WSW and showing continuity from Kameng, Subansiri and Siang districts, do not extend eastward beyond Digang river in Lohit district<sup>5</sup>. The sudden termination of these rocks appears to have resulted due to truncation by NW-SE trending Mishmi Thrust. When traced further south-eastward this thrust also cuts across NE-SW structural trends of Patkai Synclinorium and Schuppen Zone of Patkai ranges (Fig. 1).

The Mishmi Thrust has been also observed to rest over the recent alluvial sediments of the Lohit river at a locality (27° 54' : 96° 18') 12 km from Tezu in Lohit district. Here the basal part of the Mishmi Metamorphites consisting of phyllite and quartzite and dipping northeastward at moderate angle, directly overlies a recent terrace and the bed of the Lohit river. This clearly demonstrates very recent tectonic movement.

Gravity Survey in the area south of the Mishmi hills indicates that the Precambrian Crystalline Basement beneath the Brahmaputra alluvium has a northeasterly slope<sup>6</sup>. This buried basement, which is a continuation of the Peninsular shield through

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Mikir hills and Shillong plateau, shows the same general northeasterly slope right from the Mikir hills (Fig. 1).

The axial directions of Manbum folds have the same orientation (NW-SE) as that of the Mishmi Thrust (Fig. 1). They were formed subsequently as transverse fold structures to NE-SW trending folds of the Patkai Synclinorium.

the tectonic grain of the Patkai ranges, NE-SW directed phase of tectonic activity commenced as late as post-Pliocene, and appears to be active in recent time.

The Eastern Himalayan Syntaxis was formed due to bending of the Himalayan Orogenic belt around northeasterly projecting foreland of Shillong plateau which forms a promontary of Peninsular shield

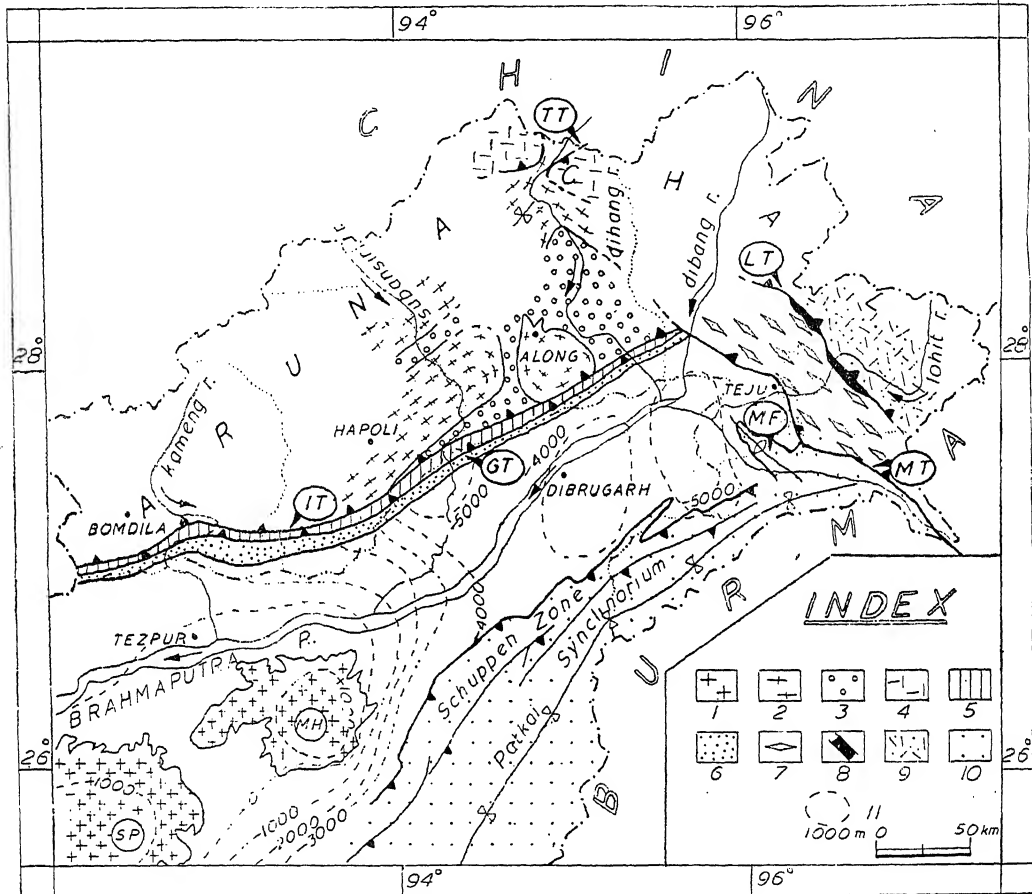


FIG. 1. Generalised geological map of Arunachal (NEFA) Himalaya and Upper Assam. Sources are, Jain *et al.*<sup>1</sup> and Evan.<sup>7</sup> 1, Precambrian Basement (Shillong Plateau—SP, Mikir Hills—MH); 2, Siang Group; 3, M'iri Group; 4, Tuting Gneissose Granite; 5, Gondwana Formation (U. Palaeozoic); 6, Siwalik Group, (U. Tertiary); 7, Mishmi Metamorphite, 8, Tiding, Ophiolite Zone; 9, Lohit Granodiorite-Diorite Body; 10, Tertiary belt of Naga-Patkai ranges; 11, Basement structure contours, after Evans<sup>7</sup>. MT—Mishmi Thrust; LT—Lohit Thrust, GT—Garu Thrust; IT—Igo Thrust; TT—Tuting Thrust; MF—Manbum Fold.

The northeasterly slope of the Basement south of the Mishmi Thrust southwestward movement of the Mishmi Thrust and NW-SE oriented Manbum folds indicate another major compressional phase of tectonic movement in NE-SW direction. As Manbum folds have affected Miocene and Mio-Pliocene rocks and the Mishmi Thrust has truncated

mass<sup>7,8</sup>. This simplified version for such a complex zone is not valid in view of our new observations discussed above—(a) the principal litho-units in western part of the syntaxis region, *i.e.*, Siang district cannot be compared with those from the eastern part of syntaxis in Lohit district, (b) the regional structural trends in ENE-WSW direction

in Siang district and NW-SE direction in Luhit district represent two different episodes of tectonic movement of the Himalayan age.

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# STEROID SYNTHESIZING CELLULAR SITES IN THE TESTIS OF THE COBRA, *NAJA NAJA* (LINN.): A HISTOCHEMICAL STUDY

IN recent years the biosynthetic pathways of steroidogenesis in the testis of the cobra, *Naja naja* have been understood as a result of identification of steroids and their *in vitro* conversions<sup>1-3</sup>. However, the cellular sites of steroid biosynthesis in the testis of this snake are yet to be determined although the steroidogenic cells in the testes of a few other reptiles have been histochemically identified<sup>3-5</sup>. The present work is designed to investigate the steroidogenic cellular sites of the cobra testis by studying the histochemical localization of  $\Delta^5-3\beta$ -hydroxysteroid dehydrogenase ( $\Delta^5-3\beta$ -HSDH),  $17\beta$ -hydroxysteroid dehydrogenase ( $17\beta$ -HSDH),  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSDH) and glucose-6-phosphate dehydrogenase by the method already described<sup>6-7</sup>. Pregnenolone,  $17\alpha$ -hydroxypregnenolone, dehydroepiandrosterone were used as the specific substrates for the demonstration of  $\Delta^5-3\beta$ -HSDH; testosterone and  $17\beta$ -estradiol for  $17\beta$ -HSDH;  $11\beta$ -hydroxy-androstenedione for  $11\beta$ -HSDH; and disodium salt of D-glucose-6-phosphate for G-6-PDH. Four mature male cobras, collected from locality around Dharwar, were used.

The specimens were in breeding period as the histological observations revealed enlarged seminiferous tubules with the cell types ranging from spermatogonia to sperm bundles and sperms. The

presence of these enzymes, indicated by the deposition of diformazan granules, was observed in the Leydig cells and to a lesser extent in the seminiferous epithelium (Fig. 1). All the four enzymes

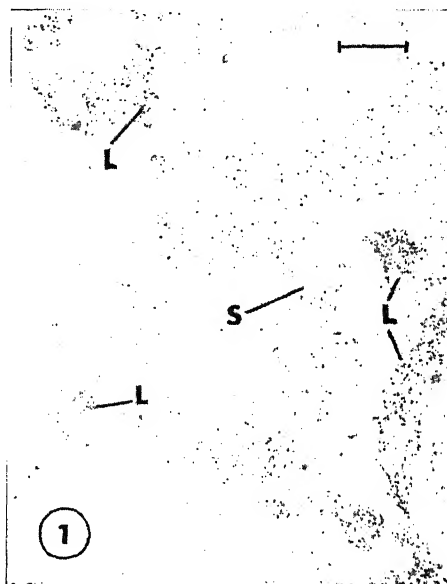


FIG. 1. T.S. of cobra testis showing intense  $11\beta$ -HSDH activity in the Leydig cells (L.). Note the weak activity in the seminiferous epithelium (S) also. The scale line indicates  $50\mu$ .

showed a common pattern of distribution. The presence of  $\Delta^5-3\beta$ -HSDH suggests the bioconversion of  $\Delta^5-3\beta$ -hydroxysteroids to  $\Delta^4-3\beta$ -ketosteroids.  $17\beta$ -HSDH has, so far, been histochemically demonstrated in the testes of chamaeleon, python and the crocodile<sup>5</sup>. The histochemical demonstration of  $17\beta$ -HSDH supports the earlier observations based on the *in vitro* studies, that the cobra testis is capable of androgen and estrogen biosynthesis<sup>1-2</sup>.  $1\beta$ -HSDH activity has not been reported in the reptilian testes. However, it is histochemically demonstrated in the testes of mouse and man<sup>6</sup>. This enzyme catalyzes the interconversions of  $11\beta$ -hydroxyandrostenedione and  $11\beta$ -hydroxytestosterone to  $11$ -ketoandrostenedione and  $11$ -ketotestosterone respectively. In the adrenal gland, it converts cortisol to cortisone<sup>8</sup>. Hence the presence of  $11\beta$ -HSDH together with  $17\beta$ -HSDH suggests that the cobra testis has the potentiality to metabolise  $11\beta$ -hydroxy-androgens as in mouse and man. Further, it is reported that the cobra testis is capable of *in vitro* synthesis of deoxycorticosterone and aldosterone<sup>1</sup> and the presence of  $11\beta$ -HSDH is an additional evidence that it is capable of corticosteroid biosynthesis. The

presence of all the four enzymes in the seminiferous tubules of the cobra testis suggests that the seminiferous epithelium is capable of steroidogenesis, hitherto known from the *in vitro* conversion studies of the isolated seminiferous tubules of *N. naja* testis<sup>2</sup>.

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#### REGIONAL DIFFERENTIATION IN THE STRUCTURE OF THE DUCTUS EPIDIDYDIS IN THE VESPERTILIONID BATS, *PIPISTRELLUS* *MIMUS MIMUS* AND *PIPISTRELLUS* *CEYLONICUS CHRYSOTHRIX*

REGIONAL structural differences in the epididymal duct have been noticed in several mammals<sup>1-7</sup>. Mansely (1959) indicated that the structural differentiation in the different segments of the duct may be in some way responsible for bringing about changes in the physiology of the spermatozoa. During the course of the study of the seasonal changes in the reproductive structures of several species of Indian bats in this laboratory, it was noticed that in every species the histology of the ductus epididymidis varies in different segments, and these differences become augmented in the sexually mature animals during the breeding season. The present paper embodies the description of the ductus epididymidis in the pipistrellid bats, *Pipistrellus mimus mimus* and *P. ceylonicus chrysothrix*. The measurements, where given, refer to *P. mimus mimus*.

In both the species the caput epididymis fits as a cap over the cranial end of the testis and is

joined by a very narrow mid-epididymis to a darkly pigmented conical cauda epididymis at the caudal end of the testis. The ductus epididymidis is a single long tube whose histology varies in different segments. However, the successive regions imperceptibly merge into one another. Since it is a highly convoluted tube, each section contains several cut ends of the duct.

In transverse sections of the caput epididymis (Fig. 1) five types of tubules can be identified.

*Type I.*—The rete tubules, which imperceptibly merge into epididymal tubules in the region between the mid and the caput epididymis, are lined with flat epithelial cells. These tubules are wide and irregular in outline, and contain practically no spermatozoa (Figs. 1 and 2).

*Type II.*—The tubules lying adjacent to the rete are more or less uniform in size being about  $75\mu$  in diameter. They are lined with tall columnar ciliated cells, 18 to  $20\mu$  in height, interspersed with a few groups of low columnar cells, 10 to  $12\mu$  in height, resulting in an irregular luminal border (Fig. 2). In addition to fine stereocilia, arising from distinct basal granules situated immediately beneath the luminal border of the cells, a few long motile cilia are also present. The lumina of these tubules contain a few spermatozoa.

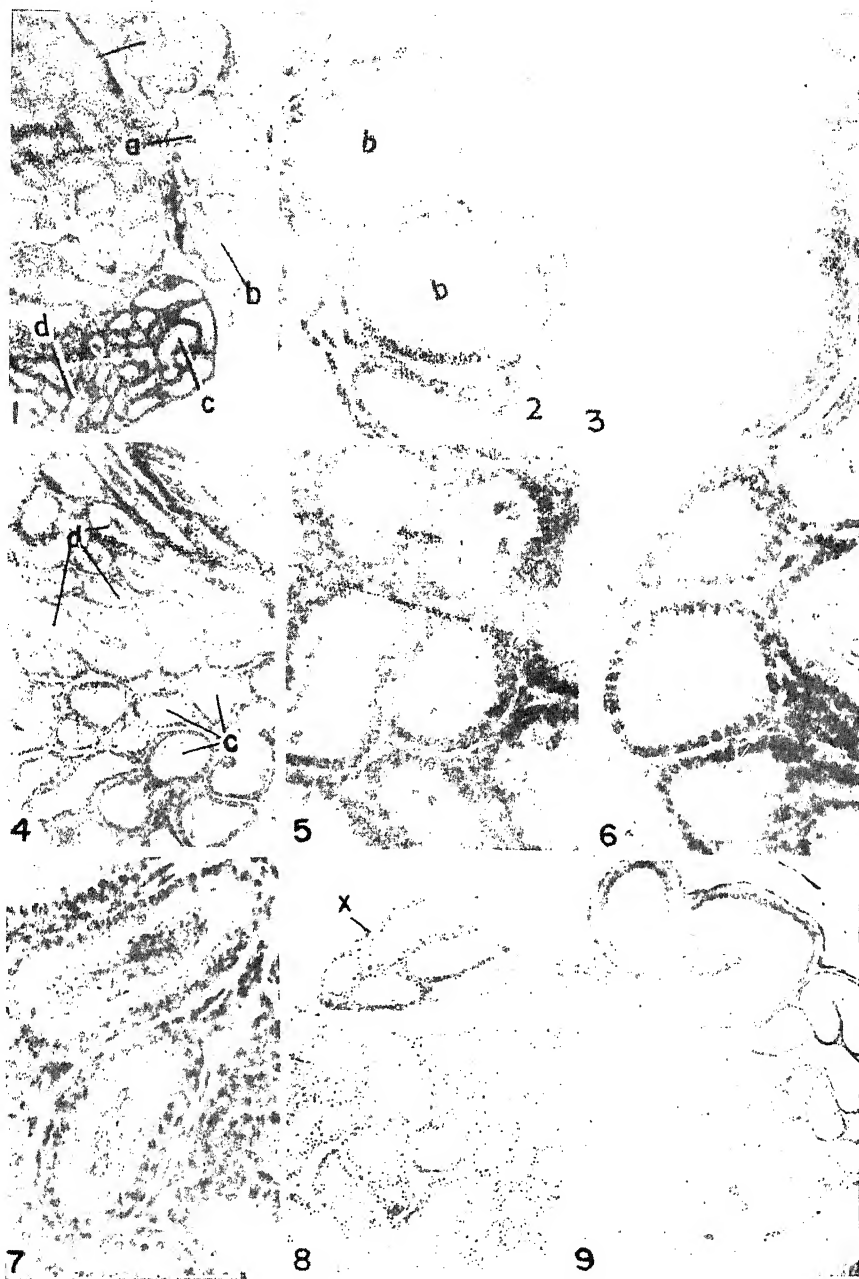
*Type III.*—These tubules are about  $65\mu$  in diameter. They are lined with tall columnar cells, and have basally situated darkly staining nuclei. A few cells get detached from the epithelium and are shed into the lumen. The lumina contain a few spermatozoa. Two types of tubules are observed in this group, (a) those with long cilia in addition to fine stereocilia and with the epithelium about 25 to  $30\mu$  in height, and (b) those with very fine stereocilia and with the epithelium about 18 to  $20\mu$  tall (Figs. 3, 4 and 5).

*Type IV.*—In this group the tubules are about  $55\mu$  in diameter, and contain a considerable quantity of spermatozoa. They are lined with closely arranged columnar epithelial cells, about 15 to  $18\mu$  in height, and have stiff stereocilia. The darkly staining rounded or oval nuclei of these cells are basally situated, but a few cells have apically situated nuclei (Figs. 4 and 6).

*Type V.*—The tubules are similar to those described under type IV, but their epithelial cells have smaller and lightly staining nuclei (Fig. 7).

The above-mentioned groups of tubules represent successive segments of the epididymal duct in the caput epididymis.

The mid-epididymis has tubules of one type, all being about  $70\mu$  in diameter and lined with cuboidal or low columnar cells, 10 to  $15\mu$  in height, and having fine stereocilia. A few basal cells with small



FIGS. 1-9. Fig. 1. Transverse section of the caput epididymis with a part of the testis of *Pipistrellus minimus mimus* showing the five types of epididymal tubules (labelled a-e),  $\times 80$ . Fig. 2. Part of Fig. 1 to illustrate the structure of the tubules of Type I (a) and Type II (b) described in the text. Note the absence of spermatozoa from their lumina,  $\times 560$ . Fig. 3. Section of the part of the caput epididymis of *P. ceylonicus chrysothrix* showing the tubules of Type III (c) described in the text. Note the well-developed stereocilia and the absence of spermatozoa from their lumina,  $\times 560$ . Fig. 4. Section of the epididymis of *P. ceylonicus chrysothrix* showing tubules of Type III (C) and Type IV (D) described in the text,  $\times 120$ . Fig. 5. Part of Fig. 1 enlarged to illustrate the structure of the tubule of Type III of *P. minimus mimus*,  $\times 560$ . Fig. 6. Part of Fig. 1 enlarged to illustrate the structure of the tubules of Type IV,  $\times 560$ . Fig. 7. Part of Fig. 1 enlarged to illustrate the structure of the tubules of Type V,  $\times 560$ . Fig. 8. Section of the mid-epididymis (x) with a part of the testis of *P. minimus mimus*,  $\times 120$ . Fig. 9. Part of the section of the cauda epididymis of *P. minimus mimus*. Note the differences in the size of the tubules. All the tubules are filled with spermatozoa,  $\times 120$ .

ovoid nuclei lie between the bases of these cells. The lumina of these tubules have a high population of spermatozoa (Fig. 8).

In sections of the cauda epididymis (Fig. 9) all the tubules have practically the same histology but they vary in their diameters from 70 to 160  $\mu$ . They are lined with cuboidal epithelium having fine stereocilia. The narrow tubules with thin muscular layer form the proximal regions of the cauda epididymis, and the wider tubules with thick muscular walls form the distal regions of the cauda epididymis. All the tubules in the cauda epididymis are densely filled with spermatozoa.

It is interesting to note that the sperm density is low in those segments of the epididymal duct where the epithelial cells are gland-like. Perhaps, the presence of these gland-like cells may have something to do with the rapid transport of the spermatozoa through these regions.

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#### **GARRA NOTATA (BLYTH) (PISCES: CYPRINIFORMES: CYPRINIDAE), A NEW RECORD FROM INDIA**

DURING the course of the investigations on the fishes of the Godavari River Basin, the author came across a species of *Garra* which agrees with the description of *Garra notata* (Blyth)<sup>1,2</sup>. This species was originally described by Blyth<sup>3</sup> as *Platycara notata* from Tenasserim, Burma. This is the second record since Blyth and first record from India.

**Material.**—One example from stream near Trimbak, Nasik District, Maharashtra, M. Babu Rao, 15-10-1973.

**Description.**—Body subcylindrical dorso-ventrally flattened towards snout and laterally flattened towards tail. Dorsal profile is arched; it rises from the tip of the snout to the base of the dorsal fin, beyond which it slopes down to the base of caudal fin. Ventral profile straight and more or less horizontal throughout. The undersurface of the head and body are greatly flattened. Eyes are situated in the middle of head length, lateral, but invisible from

below. Inter-orbital region somewhat convex. Snout smooth, rounded, projecting beyond mouth. Mouth transverse, semicircular and inferior; lips continuous, covered with anterior and posterior labial folds; a suction disc behind lower lip consisting of semicartilaginous pad. Two pairs of barbels, the rostrals thread-like nearly as long as the diameter of the eye, while the maxillary much smaller. Pectorals originate just after the operculum from the ventral side. Ventrals originate after some distance from the tip of pectorals and from below the base of

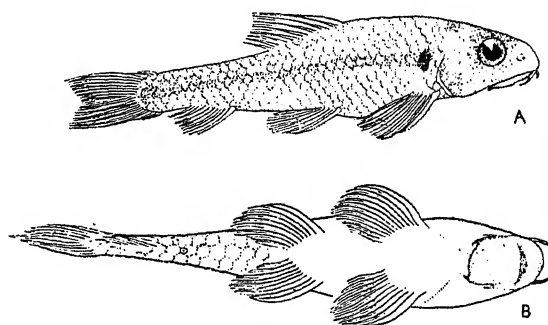


FIG. 1. *Garra notata* (Blyth). A. Lateral view, B. Ventral view.

dorsal fin. Origin of dorsal nearly equidistant from the tip of the snout and the base of the caudal fin. Anal origin after some distance from the vent. Scales deciduous in preserved specimen. Colouration, dark brown above and whitish below. A black band along the lateral line becoming prominent towards the tail. A black spot near the angle of the operculum. Base of the dorsal fin dark.

**Measurements (in mm.).**—Total length 35.3, standard length 28.0, head length 8.0, body depth 5.5, eye diameter 2.5, snout 3.5, interorbital 3.5, prepectoral distance 7.5, preventral distance 16.0, predorsal distance 14.2, pectoral fin length 7.0, ventral fin length 5.2, dorsal base 4.5, anal base 2.5.

Pectoral f.r. 13, Ventral f.r. 9, Dorsal f.r. ii + 9, Anal f.r. i + 6, Caudal f.r. vii + 19 + v.

**Remarks.**—The occurrence of this species in Tenneserim in Burma and again in the Western Ghats indicates discontinuous distribution. Menon<sup>2</sup> explained the present-day distribution of the various species of *Garra* as due to a series of evolutionary waves of migration from the ancestral home, viz., Southwest China (Yunnan). It appears during the southward migration *G. notata* must have reached Burma and during one of the westward migrations the species has reached the northern region of the western ghats. With time, the intermediate region might have been replaced by the other species of the genus restricting the distribution of this species in the

present day in these two regions. The fact that it is a relic species<sup>2</sup> gives support to this presumption.

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ON THE OCCURRENCE OF *CIRRATULUS*  
*CIRRATUS* O. F. MÜLLER (POLYCHAETA:  
CIRRATULIDAE) A NEW RECORD FOR  
INDIAN WATERS

SEVERAL species of polychaetes mainly belonging to families Serpulidae and Sabellidae have been reported as important fouling groups from different regions in India. In addition to these sedentary species, many polychaete worms have also been found to occur in mud bottom<sup>1-3</sup>, as surface foulers on underwater surfaces or in association with major fouling<sup>4</sup> and wood boring organisms<sup>5</sup>.

In the course of our investigations on the occurrence and distribution of marine fouling organisms, many of the experimental panels were observed to be covered by a large number of the polychaete *Cirratulus cirratus*. The habitat of the animal appears to be the protected spaces in between the shells of fouling animals and crevices of oyster and other molluscan shells. They have also been encountered in empty barnacle shells normally filled with mud and detritus. In a few instances, the animals were found residing in hollow spaces, below oyster shells but they do not appear to have any boring abilities.

The animals are bright red in colour when removed fresh from the panels. Length of the animal varied from 10 mm to 60 mm (Fig. 1).

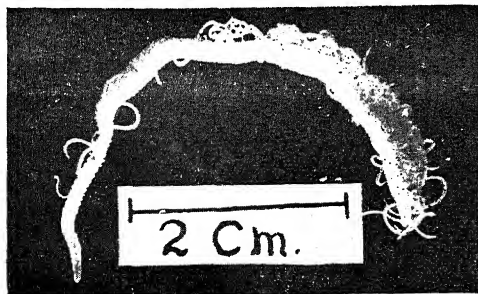


FIG. 1. *Cirratulus cirratus* O. F. Müller. Entire animal showing gills all over the body.

The prostomium is long and annulated. It is blunt-conical with an oblique row of 4 to 8 small eye spots on either side. The first setigerous segment carries a transverse row of tentacular filaments. The red gill filaments also commence from the same segment. Both the tentacular and gill filaments are interlocked with each other and the detached filaments appear like small independent worms. It has been suggested that tentacular filaments are really prostomial tentacles which have shifted backwards in position<sup>6</sup>. The gills are present throughout the body. Segments are distinct and similar throughout the body, with capillary setae situated on either side in two bundles. Dorsal and ventral acicular setae are, however, absent on some of the anterior segments.

The characters of the specimen examined closely resemble the characters described for *C. cirratus* by Fauvel<sup>1</sup> and the polychaete is hence identified as *Cirratulus cirratus* O. F. Müller. The animal is believed to be cosmopolitan in its distribution<sup>1</sup> and has been reported from Japan, Indochina, Persian Gulf, Atlantic, Arctic and Antarctic Oceans; the species however was not recorded from Indian waters. The present report on the occurrence of *C. cirratus* from Visakhapatnam is, therefore, a first record of the species from India.

The authors are thankful to Captain P. R. Sen, IN and Sri S. V. S. Rao, Director and Deputy Director of this laboratory respectively, for their constant encouragement given during the course of present studies. The authors are also indebted to Dr. R. Philip Dales, Professor of Zoology, Bedford College, London, for identification of the specimen.

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### COLORIMETRIC ESTIMATION OF SESAME OIL IN ADULTERATED SAMPLES

SESAME oil is sometimes adulterated with other cheaper oils like castor and nigerseed, etc. For the qualitative detection of sesame oil, Baudouin test or the modified Villavecchia-Fabris test<sup>1</sup> is often used. Desai and Patel<sup>2,3</sup> have reported the use of Ever's modified Bellier test to determine the percentage of adulteration of sesame oil with groundnut or nigerseed oils. In the present communication, we are reporting a general colorimetric method for the estimation of sesame oil in adulterated samples. The Villavecchia-Fabris test is suitably modified and used for the estimation.

To prepare the known adulterated samples of different concentrations of the sesame oil, benzene solutions (4% v/v) of sesame oil and nigerseed oil were mixed in suitable proportions. 5.0 ml of the benzene solution is run down from a burette into a 25 ml separating funnel, to which were added 10 ml of concentrated hydrochloric acid (sp. gr. 1.18) and 0.2 ml of 2% alcoholic solution of freshly distilled furfural and shaken exactly for one minute. The mixture is then allowed to settle, the acid layer is withdrawn and the optical density is measured exactly after 10 minutes of the addition of furfural solution using Ilford No. 621 (filter No. 1), (370–515 m $\mu$ ) of Unicam Sp. 1300 colorimeter (taking con. hydrochloric acid as blank). A calibration curve is then plotted between concentration of sesame oil and optical density (Fig. 1). The calibration curve is composed of two straight lines and can be used to estimate the concentration of sesame oil from 6 to 100%.

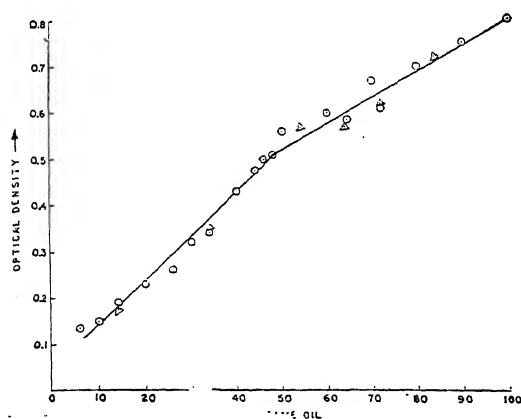


FIG. 1

The calibration curve obtained above has been used to estimate the concentration of sesame oil in samples adulterated with different oils. These samples were prepared in the same manner as in

the case of preparation of calibration curve. The optical densities of the acid layers were determined as described earlier. The sesame oil content of the samples was read directly from Fig. 1. (The corresponding points are marked with  $\Delta$  on Fig. 1). The results obtained for different adulterant oils are expressed in Table I.

TABLE I

Sl. No.	Adulterant oil	% Sesame oil (known)	% Sesame oil derived from Fig. 1	% error
1	Nigerseed	84.0	85.5	+1.78
2	Groundnut	64.0	58.5	-8.59
3	Castor	54.0	58.5	+8.33
4	Soyabean	34.0	32.0	-5.88
5	Mineral oil (liquid paraffin)	14.0	13.5	-3.57
6	Mustard	72.0	68.0	-5.55

It is evident from Table I that the method can be used to estimate sesame oil in adulterated commercial samples within reasonable limits of error. The above method can also be used for estimating the quantity of sesame cake in adulterated oil cakes (obtained from expellers and not by solvent extraction) commonly used as cattle feed. The cake sample can be extracted with hexane, and the oil obtained is subjected to the above method to determine the sesame oil content in it and hence the quantity of sesame cake in the sample.

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Regional Research

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### EFFECT OF CERTAIN RARE EARTH ELEMENTS ON THE REGULATION OF STOMATAL MOVEMENTS IN *ASPHODELUS TENUIFOLIUS* CAV.

A NUMBER of workers have studied the role of certain metallic cations and growth regulators<sup>3,5,7,8</sup>. Besides growth regulators and metallic cations, the rare earth elements should also have some effect on the regulation of stomatal movement. In the present study, the effect of some rare earth elements on the regulation of stomatal movement of *Asphodelus tenuifolius* has been studied.

TABLE I

Effect of incubation in Paraseodymium chloride on the stomatal regulation in isolated peelings of *A. tenuifolius*

Concentrations in M	Incubation for 3 hours		Incubation for 24 hours	
	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test
0	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+
0.1	0.0 $\pm$ 0.0	+	0.0 $\pm$ 0.0	+
0.05	2.7 $\pm$ 0.6	Slight	0.0 $\pm$ 0.0	+
10 <sup>-2</sup>	3.75 $\pm$ 0.7	—	6.9 $\pm$ 0.7	—
10 <sup>-3</sup>	3.3 $\pm$ 0.6	Slight	5.4 $\pm$ 0.7	—

TABLE II

Effect of spray treatment with Paraseodymium chloride on the stomatal regulation in *A. tenuifolius*

Concentrations in M	After 24 hours		After 72 hours		After 120 hours	
	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test	Pore width in $\mu$	Starch test
0	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+	3.4 $\pm$ 0.6	+
0.01	3.4 $\pm$ 0.6	+	8.1 $\pm$ 1.3	—	12.3 $\pm$ 2.0	—
0.5	0.0 $\pm$ 0.0	+	0.0 $\pm$ 0.0	+	2.5 $\pm$ 0.6	+

The general technique employed in the present study has been fully described in a previous paper<sup>7</sup>. Besides isolated epidermal peelings the experiments were also performed with plants growing in the field. The isolated peelings were incubated in different concentrations of Paraseodymium chloride (0.05, 0.01, 10<sup>-3</sup> and 10<sup>-4</sup> M) and in distilled water for control. The observations were taken after 3 and 6 hours of incubation.

For intact plants, the solutions were sprayed only once and the observations were taken after 24, 72 and 120 hours of spray treatment. Only two concentrations (0.5 and 0.01 M) were used in these experiments, with 0.01% Triton as surfactant.

The effect of Paraseodymium chloride on stomatal regulation in isolated peelings has been shown in Table I. It is evident from Table I that the maximum opening (7.0  $\mu$ ) was found in peelings incubated in 10<sup>-2</sup> M solution, when observed after 24 hours. In higher concentrations plasmolysis in epidermal cells took place resulting in the closure of stomata. A negative test for starch was observed in open stomata.

In intact plants the maximum opening (12.3  $\mu$ ) was found after 120 hours of spray treatment, after which it started reducing (Table II). A positive test for starch was observed in open stomata. In higher concentrations the stomata closed down due to plasmolysis (Table II).

It is evident from the foregoing observations that rare earth elements have also some role on the regulation of stomatal movement in *A. tenuifolius*. Some workers have already reported that only monovalent ions are responsible for opening of stomata<sup>6</sup>. Bivalent and trivalent ions have also been shown to effect stomatal opening<sup>2</sup>. All these metallic cations invariably cause the hydrolysis of guard cells starch, which helps in the opening of stomata<sup>1,2,4,6,8</sup> and similar results were also observed with rare earth elements in the present study. The effect of Paraseodymium chloride is slower on intact plants. Thus, it appears that not only monovalent, divalent and trivalents but also rare earth elements can cause the opening of stomata.

Thanks are due to Shri V. S. Rajpurohit of Chemistry Department for the gift of some rare earth elements and also to the Head, Botany Department, for facilities.

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#### A METHOD FOR THE DETERMINATION OF KERATINOPHILIC MOLDS

KERATINOPHILIC fungi in general are potential danger to man and animals. These fungi can be isolated from the soil with the help of baits consisting of keratinized substrates like hair, feathers, hoofs, nails, etc. Griffin<sup>2,3</sup> noted the initial colonization of sterile hair by a succession of molds which often did not show keratinolytic properties. These were the fast growing fungi whose spores were

abundant in the soil. On the other hand, the fungi which possess the ability to utilize the keratin of the hair as sole source of nutrient or capable of attacking and digesting hair keratin can be confirmed by the method described here.

The organisms isolated from the colonized hair were cultured on the Sabouraud dextrose agar medium. To evaluate the keratinolytic property, the individual isolate was grown on a sterile hair tied on a glass rod bent to a particular shape A as shown in Fig. 1. Hair was inoculated in the middle of its length simply by transferring the fungal spores or mycelial bits with the help of a sterile needle. The glass rod was suspended with the help of a cork in a large specimen tube having sterile moist cotton D at the bottom. To reduce the loss of water a small quantity of glycerine was mixed with it. The whole assembly was sterilized prior to inoculation with the fungus. After 20 to 25 days of incubation at 26°C (temperature and incubation period can vary according to the test organism) the test fungus developed a colony (Fig. 1 C) on the hair and if growth is continued the hair would break, indicating the digestion of the keratin of the hair and its disintegration by the test fungus.

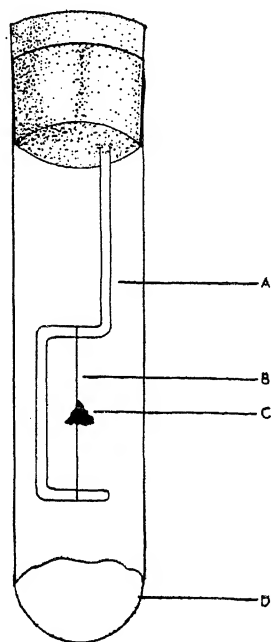


FIG. 1. Growth of test fungus (Keratinophilic) on hair. A, glass rod; B, hair; C, fungal colony; D, moist cotton.

Most of the fungi which attacked keratin containing materials were capable of forming

fronded mycelium and boring hyphae<sup>1</sup>. During the study of keratinophilic fungi from a variety of soils, a large number of fungi which were isolated from the buried hair were found nonkeratinolytic when tested by the above method. This technique can be applied to determine the ability of a fungus to decompose different types of hairs, i.e., human horse, cattle, etc. Keratin decomposing capacity of various keratinophilic fungi can also be assessed by this method.

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#### PROANTHOCYANIDINS IN SEEDS OF THE LEGUMINOUS WEED, *RHYNCHOSIA MINIMA*

DURING seed germination studies on *Rhynchosia minima* we invariably observed the formation of an unusually prominent brownish black halo around each seed sown on moist filter paper discs. We presumed it to be due to leaching by seeds of a large amount of phenolic substances. Therefore, we undertook studies (i) to confirm the presence of phenols in the seed material, (ii) to extract and identify the phenolic substances, and (iii) to find out the effects, if any, of the seed extract on seed germination.

**Experimental Material.**—*Rhynchosia minima* is a leguminous twiner. It bears 1–3 seeds a pod. The seeds are sub-reniform; their average size is 3.35 mm along the longitudinal axis, and 2.5 mm across the widest region. They have a very hard texture; their surface is inconspicuously mottled black and white.

**Seed Germination.**—Seeds were sown on filter paper discs moistened with all-glass distilled water in petri plates. Each petri plate culture contained 9 seeds. Each experiment was replicated thrice with 6 cultures a replicate. All cultures were maintained in light (800–1,000 lux) at  $25 \pm 2^\circ \text{C}$  for 5 to 10 days, and were periodically irrigated with distilled water. The filter paper discs were not renewed any time during the entire culture period.

Untreated seeds did not germinate; a mere scarification of seeds with sand paper did not also induce germination. Germination occurred only if the

seeds were mechanically damaged; a random pin-pricking of the seed surface brought about 70% germination 3 days after sowing. Pin-pricking the hilum caused almost 100% germination 2 days after sowing. A very prominent halo was formed around each seed within a day of sowing. No microbial infection ever appeared either on the seed or in the halo.

**Isolation of Phenolic Compounds.**—To test the presence of phenols in seeds of *Rhynchosia minima* we treated the seeds with an aqueous solution of an antioxidant, ascorbic acid (5,000 ppm) and then studied their germination. As expected, no halo appeared around any ascorbic acid-treated seed during germination. Also, if the seeds were dehusked 12 hr after soaking them in distilled water, only a light brown halo was formed. This proved that the phenolic substances responsible for halo formation are largely resident in the seed coat. Therefore, powdered seeds (25 g) were extracted 3 times with 30 ml portions of methanol in the cold. The combined extracts were concentrated to 30 ml, filtered, and then diluted with dry ether. A white precipitate was formed. After centrifuging, the precipitate was redissolved in methanol and reprecipitated by dry ether; reprecipitation was repeated twice. Finally, a white semi-crystalline solid (200 mg) was obtained. It was sparingly soluble in ethyl acetate and acetone, but readily soluble in water and methanol. With dilute sodium hydroxide it readily gave a brown solution which darkened rapidly. Paper chromatographic behaviour indicated the substance to be homogeneous. The following reactions demonstrated its proanthocyanidin nature: (i) Tetradiazotised benzidine gave a deep red colour, showing the phenolic nature of the substance, (ii) Boiling in ethanolic hydrochloric acid or in butanolhydrochloric acid gave a deep red-pink colour, (iii) Ehrlich reagent<sup>1</sup> gave a red-violet colour, and ethanolic cinnamaldehyde-HCl<sup>2</sup> a deep red colour, both tests indicating the presence of unconjugated phloroglucinol nuclei, (iv) Aqueous ammonium molybdate gave a deep yellow colour indicating the presence of catechol nuclei.

**Anthocyanidin Formation.**—The proanthocyanidin (10 mg) was boiled with 10% ethanolic hydrochloric acid for 1 hr. A deep red solution was formed. The anthocyanidin chloride was isolated; it was found to agree with authentic cyanidin chloride on paper chromatograph. Its ethanolic hydrochloric acid solution showed absorption maxima at 544 nm.

The proanthocyanidin may therefore be regarded as derived from leucocyanidin. All the mother liquors (methanol-ether) left after removing the

proanthocyanidin were pooled and the solvent evaporated completely. The residue contained either soluble simpler phenolic compounds and other substances; further examination has indicated the presence of gallic acid, protocatechuic acid, and certain other phenolic compounds.

**Role of Proanthocyanidins.**—Leucoanthocyanidins have been reported to affect plant growth and development. For example, leucocyanidin, injected into young radicles of the broad bean variety Giant White Windsor, at a concentration greater than 2.99 ppm considerably retarded root development, whereas at lower concentrations it stimulated the root development<sup>3</sup>. However, in our studies treatment of seeds of *Rhynchosia minima* with the crude extract of proanthocyanidins in a wide range of concentrations from 1 ppm to 100 ppm neither promoted nor inhibited seed germination. Thus, although leucoanthocyanidins have been isolated from more than 30 species belonging to 28 families of flowering plants<sup>4</sup>, and in *Rhynchosia minima* (present work), the role of leucoanthocyanidins in plant growth and development has yet to be unequivocally demonstrated.

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#### DRUSES IN THE OVULAR ENVELOPES OF EUPHORBIACEAE

BESIDES the structure and development of the integuments in angiosperms, considerable attention has been paid to their cell inclusions in the classical as well as modern embryological works. So far, there were records of the occurrence of chlorophyll, chromoplasts, anthocyanin, oil, starch grains, fats and rarely bacteria and fungi in the ovular envelopes of angiosperms belonging to the various families of Dicotyledons and Monocotyledons<sup>1-2</sup>.

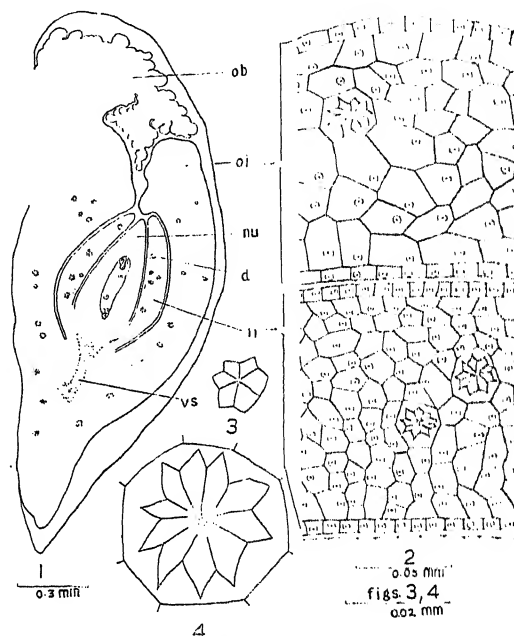
But the occurrence of druses (star-shaped calcium oxalate crystal deposits) in the ovular envelopes of angiosperms has not been reported so far as evidenced by a scrutiny of various morphological, anatomical and embryological works<sup>1-10</sup>. Scott

(1941)<sup>11</sup> mentioned that calcium oxalate is rarely observed during the development of the seed in *Ricinus communis* but for one or two small crystals in the caruncle and not elsewhere, whereas Singh's (1954)<sup>12</sup> account of the seed structure in the same species made no mention about their occurrence at any stage of the seed development.

During a comparative study of the development of ovule in several taxa of the Euphorbiaceae<sup>13</sup>, it has been presently found that in *Trewia nudiflora* Linn., a liberal distribution of druses occurs in both the integuments of the ovule, a feature recorded for the first time in Euphorbiaceae in particular and angiosperms in general. Here, the ovary is 2-4 carpellary with as many loculi. There is a single anatropous, bitegmic, crassinucellate ovule in each loculus on axile placentation. There is a massive placental obturator. The micropyle is formed by both the integuments. The outer integument is 6-12 layered and the inner 10-15 layered. The outer integument is broader at the apical end. The outer and inner epidermal cells of both the integuments are smaller and more compact than the inner cells.

The occurrence and distribution of druses in the various stages of the ovule development has been studied. In the very young ovules no druses have been observed in any part of the ovule. During the older stages of development of the ovule, especially from the mature embryo sac stage onwards, a liberal occurrence of druses has been noticed in both the integuments and also in the tissue of the ovule below the vascular strand in the chalazal region (Figs. 1, 2). They are not found in the outer and inner epidermal cells of both the integuments, obturator, nucellus or in the embryo sac. Different developmental stages of druses are often found in the same integument. During the early stages of crystal formation, the druses are observable as minute specks of light under the oil immersion of the compound microscope. Later, due to gradual accretion of calcium oxalate the crystals show a dendritic growth and ultimately assume their characteristic stellate appearance (Figs. 3 and 4). Microchemical tests made by us using a saturated solution of cupric acetate<sup>14</sup> confirmed that these crystals are of calcium oxalate. Banerjee and Dutt (1944)<sup>15</sup> working on the same species somehow missed to record this feature. Material used in the present study, obtained incidentally from three different localities, namely: (a) Waltair in Visakhapatnam District, A.P.; (b) Amalapuram in East Godavari District, A.P. and Indian Botanic Gardens, Howrah, invariably showed the occurrence of the druses in the integuments of *Trewia nudiflora*. Since calcium

oxalate crystals and other ergastic substances played a significant role as additional evidence in the systematic studies of various other taxa<sup>8</sup>, the same needs a critical appraisal in Euphorbiaceae.



FIGS. 1-4. Fig. 1. L.S. ovule at mature embryo sac stage. Fig. 2. L.S. portion of integuments showing druses in their cells. Figs. 3 and 4. Young and full formed druses.

ob—obturator, ii—inner integument, d—druse, o—outer integument, nu—nucellus, vs—vascular supply.

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#### GENETICAL STUDIES IN RICE-BRITTLENESS AND EASY-SHELLING HABIT

STRUCTURAL modifications leading to morphological variations have been reported in rice and include changes in one or more plant parts. Siddiq (1967) reported a brittle culm mutant in which the kernels get easily detached from the enclosing outer husk on slight pressure.

In the present investigation, two strains exhibiting brittleness were crossed to study the allelic relationship. The extract 71/R3 with easy-shelling habit derives its gene for brittleness from an American variety CI 7392. Easy shelling habit is different from "easy threshability" or "shattering habit" meaning easy detachment of the spikelets or grains from the panicle. The second parent is a derivative from a cross between a Japanese marker H.153 and an *Indica* variety, N.22. The cross was made in 1972 (*kharif* season) and the  $F_1$ ,  $F_2$  and  $F_3$  generations were studied in 1973 and 1974.

Brittleness of plant parts was noticed in the  $F_1$  generation and all the  $F_2$  plants had this character.

The  $F_1$  plants did not exhibit easy-shelling nature of the grain and in the  $F_2$  generation, 228 plants were normal and 192 had easy-shelling habit giving a ratio of 9 normal : 7 easy-shelling ( $\chi^2 = 0.60$ ;  $p$  value between 0.50 to 0.30). The ratio was subsequently confirmed by the behaviour of lines in the  $F_3$  generation. It was therefore concluded that two recessive genes control the easy-shelling nature.

Siddiq (1967) reported that easy-shelling habit was closely associated with brittleness. Both parents used in the present study have the brittle character but easy-shelling habit was noticed only in 71/R.3. Based on the present data and available

information it is inferred that (i) the genes for brittleness vary in their effect and (ii) the genes for brittleness in the parents are probably located very close to each other.

Brittleness can express itself at an early stage and/or at flowering or even later. In some varieties, the parts remain brittle at all stages. The culm or the leaf or both may be brittle; in extreme cases the panicles may also be brittle.

Singh (1971) suggested three sub-units in the brittle gene locus to explain differences in the character expression. He also postulated a suppressor gene locus. From the present studies, along with the data already available, it is inferred that the genes for brittleness are probably situated very close to each other and that one or more of the genes controlling brittle character are also responsible for the easy shelling nature. Another possibility would be that the genes for easy-shelling and brittleness are independent but closely linked in at least some varieties.

All the grains in the panicle did not exhibit the easy-shelling habit; the percentage never exceeded 75. Easy-shelling habit would result in increased hulling recovery, but, the character is not economically useful since it is associated with brittleness of the plant parts.

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#### SOME NEW FRUIT ROT DISEASES

DURING a survey of local markets some storage diseases of fruits and vegetables were noticed. These were fairly common and were responsible for considerable loss.

In the month of June and July 1972 ripe fruits of *Mangifera indica* L. var. 'Langra' available in the market exhibited a dry rot. It made its appearance in the form of a black spot, circular or oval in shape. The spot enlarged as the disease progressed. The infected fruits shrivelled and ultimately dried up (Plate I). Isolations from the diseased fruits were made and *Boothiella tetraspora* Lodhi and Mirza was isolated. The

disease was quite prevalent and it was not confined to any particular location on the fruit but was capable of appearing anywhere on the fruit. Considerable loss to mango ('Langra' var.) fruits in storage occurred as a result of the disease.

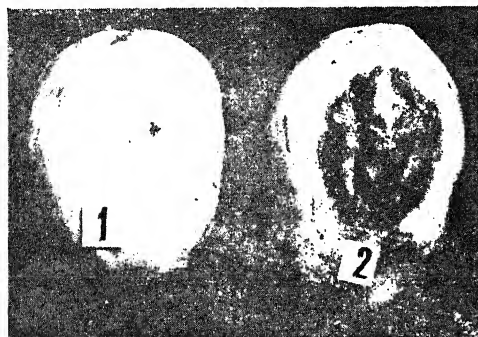


PLATE I. 1, Healthy fruit of *M. indica* L. var. 'Langra'; 2, Diseased fruit of *M. indica* L. var. 'Langra'.

In February and March 1973 fruits of *Carica papaya* L. were found to be infected by *Neurospora crassa* Shear and Dodge. The fungus caused a soft rot of papaya fruits. Infected areas became discoloured, soft and pulpy. The disease could appear anywhere on the fruit and in severe infection the entire fruit became involved and exhibited the rot.

*Cordia dichotoma* Forst f. is an important vegetable crop of summer season. Its berry-like fruits are edible. A dry rot of its fruits was discovered and was found to be caused by *Thelavia terricola* (Gilman and Abbott) Emmon. The disease was quite common during summer months. Small brownish lesions, oval to irregular in shape, appeared on the fruits. These enlarged slowly, ultimately producing a dry rot (Plate II) which was always confined to the equatorial region of the fruit, the stalk and the calyx ends of the fruits were not affected by the disease. The disease damaged the fruits only during storage and transit.

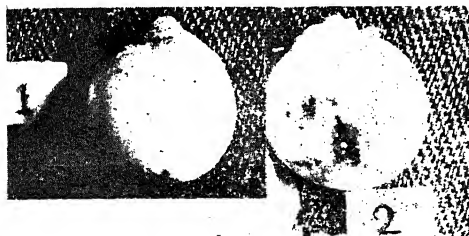


PLATE II. 1, Healthy fruit of *C. dichotoma* Forst f.; 2, Diseased fruit of *C. dichotoma* Forst. f.

The pathogenicity of the above organisms was established as Koch's postulates were fully satisfied.

The spore sizes and the general characters of different pathogen included in the present study were similar to the type species and hence those characters have been omitted.

The pathogens are being reported for the first time on these hosts.

Sincere thanks are due to Prof. D. D. Pant, for providing laboratory facilities and to the Director, C.M.I., Kew, England, for kindly identifying the organisms.

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#### ACTIVATION OF SUCCINIC DEHYDROGENASE ACTIVITY OF THE SHOOT, RESPIRATION AND PROTEIN SYNTHESIS OF SEEDLINGS OF *PHASEOLUS RADIATUS* L. BY B VITAMINS

KNOWLEDGE of the relation of vitamins to higher plants is still far from complete. However, in recent years importance has been given to the association of B group vitamins with growth and yield of crop plants<sup>1</sup>. The effect of B<sub>6</sub> (pyridoxin) on pea and wheat seedlings<sup>2</sup> and of nicotinic acid on growth, development, productivity and changes in proteins and nucleic acids were studied<sup>3</sup>. The present investigation was undertaken to study the effect of biotin, pyridoxin, niacin and thiamine on succinic dehydrogenase, respiration, endogenous vitamin contents and protein content of the green gram.

The study was undertaken with the variety G.G. 525 of *Phaseolus radiatus* L. The seeds were germinated in sterilized petridishes. On the third day the root system of the seedlings was washed with sterile water and the seedlings were transferred to fresh and sterilized petridishes containing 10 PPM of the vitamins (critical concentration), as lower concentrations were ineffective while higher concentrations were toxic. The vitamin treatment was given only for 24 hr to avoid the possibility of infection and then they were allowed to grow in distilled water in luminosity of 2,000 Lux. The protein content of the seedlings was estimated according to the method of Lowry *et al.*<sup>4</sup>. The succinic dehydrogenase activity was determined using the method of Lee and Lardy<sup>5</sup>. Pyridoxin (B<sub>6</sub>) and thiamine (B<sub>1</sub>) were estimated by using colorimetric methods described by Winton and Winton<sup>6</sup>. The seedlings were cut into bits and the respiration was measured from oxygen consumption in a Warburg apparatus.

On the fourth day of seedling growth, the succinic dehydrogenase activity of the shoot portion was more with the vitamin treated sample than with

TABLE I

Days after sowing		Control	Biotin	Niacin	Pyridoxin	Thiamine
4 days	SDH { Root	2.163	1.013	0.997	1.330	0.981
	{ Shoot	0.731	2.041	1.500	2.212	2.435
	O <sub>2</sub> uptake $\mu$ /seedling/hr	57.28	23.39	36.14	38.73	27.93
	Protein content mg/seedling	3.765	2.758	3.785	2.674	2.673
5 days	Length in cm	15.70	13.05	15.30	14.95	16.00
	SDH { Root	1.256	0.480	1.086	0.818	0.271
	{ Shoot	0.347	0.258	0.170	0.247	0.499
	O <sub>2</sub> uptake $\mu$ /seedling/hr	45.19	35.32	52.72	55.46	48.53
6 days	Protein content mg/seedling	1.836	2.253	6.208	2.248	2.415
	Length in cm	18.00	16.90	16.60	17.45	17.75
	SDH { Root	1.523	1.480	1.900	0.899	1.357
	{ Shoot	1.048	0.627	0.821	0.515	0.558
7 days	O <sub>2</sub> uptake $\mu$ /seedling/hr	44.46	34.59	35.16	37.76	41.88
	Protein content mg/seedling	0.715	0.265	0.855	0.626	0.390
	Length in cm	17.40	19.00	17.60	18.90	18.60
	SDH { Root	1.200	1.654	1.825	1.216	1.095
	{ Shoot	0.873	1.297	1.400	1.137	1.388
	O <sub>2</sub> uptake $\mu$ /seedling/hr	36.00	29.00	34.75	23.12	29.00
	Protein content mg/seedling	1.608	1.599	1.619	1.678	1.326
	Length in cm	21.50	20.90	20.70	21.20	19.50

Note: SDH (Succinic dehydrogenase) expressed as  $\mu$  moles/mg protein/hour.

TABLE II

Endogenous levels of B<sub>1</sub> and B<sub>6</sub> in green gram treated with B<sub>1</sub> and B<sub>6</sub>  
(Vitamin content as  $\mu$ g/gm dry weight)

Treatment	Vitamin	Days after sowing			
		4th day	5th day	6th day	7th day
Control	B <sub>1</sub>	1211 (17.2)	1155 (16.5)	1311 (14.8)	1290 (14.5)
	B <sub>6</sub>	387 (5.5)	470 (6.4)	587 (7.4)	691 (7.9)
Thiamine	B <sub>1</sub>	1014 (13.8)	1067 (14.1)	1022 (11.5)	982 (11.4)
Pyridoxin	B <sub>6</sub>	372 (5.0)	436 (5.9)	577 (7.1)	663 (8.2)

Note: The figures in the parentheses represent vitamin values as  $\mu$ g/seedling.

the control (Table I). In general the enzyme activity of the shoot was less than that of the root. The activation of the enzyme activity on the fourth day resulted in an increase in the respiration and in the protein content of the seedlings on the fifth day. An increase in respiratory activity, chlorophyll and protein contents with riboflavin treatment was earlier reported by Gopalarao<sup>7</sup>. In the present study, the temporary activation of succinic dehydrogenase activity of the shoot on the fourth day was followed by a reduction in its activity both in root and in shoot portions. The possible reason for reduction in the activity of the enzyme and respiration may be due to lower endogenous levels

of vitamins (thiamine and pyridoxin) which were not probably sufficient to cause an increase in the respiratory activity as coenzymes (Table II). Williams, Eakin and Shive<sup>8</sup> suggested that it is possible for a vitamin (when exogenously applied) to act as an inhibitor of its own coenzyme. The present study, in which the endogenous levels of vitamins were lowered, due to exogenous supply supports the above suggestion. Growth of the seedlings (elongation) was not affected by vitamin treatment (Table I).

The authors thank Professor V. S. Rama Das for providing facilities.

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# FREAK 'FLOWERS' FROM THE INVOLUCRE OF BRACTS IN SUNFLOWER (*HELIANTHUS ANNUUS* LINN.)

REPORTS on structural abnormalities in flowers (organoid galls) and spontaneous occurrence of freak flowers have been many (Bond, 1945; Giriraj and Swamy Rao, 1973; Kempanna, 1969; Sundaramurthi and Sivagnanam 1965; Sivagnanam *et al.*, 1961). The authors report herein a rare organoid gall in Sunflower. In a crop of Sunflower grown over an area of three hectares, one of the plants in the border rows showed a capitulum from which the involucre of bracts differentiated into both ray and disc florets (Figs. 1, 1a and 2). The

the bracts with ray florets had atrophied corollas and abortive pollen grains. In rose, sepals of a flower have been seen to develop into structures much like a leaf (Bond, 1945). Similarly in *Eriophyces*, stamens or carpels have been observed to change into petal-like structures (Sinnott, 1960).

The origin of such freak flowers and structural abnormalities is possibly due to a hormonal imbalance or failure of the hormonal systems (Heslop-Harrison, 1952).

The authors wish to express their thanks to Dr. A. Seetharam, Geneticist, Sunflower Scheme (AICORPO), for providing the necessary facilities.

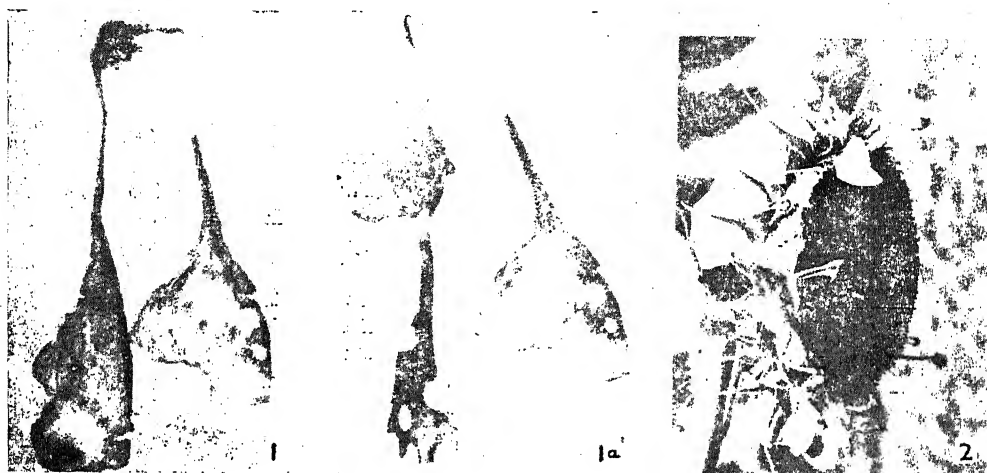


FIG. 1. Development of ray floret (atrophied) on the bract along with a normal bract.

FIG. 1a. Development of disc floret on the bract along with a normal bract.

FIG. 2. Development of ray and disc florets from the involucre of bracts on the capitulum.

apical tips of these abnormal bracts were observed to be flattened which then elongated and differentiated into a shield from which either the ray floret or disc floret developed. These florets developing from such bracts were enclosed in miniature bracts which were three in number in both the cases. During the nascent period of its development, the tips of these bracts resemble unopened florets of a young capitulum. This phenomenon was observed in a number of bracts of the second whorl of the involucre. Compared to this, the other whorls of the same involucre were quite normal. The florets on these bracts have their origin from a pad-like structure of the elongated apical tip. The florets on these pads were surrounded by three miniature bracts. The disc florets on these bracts were quite normal with a well-defined stigma and ovary, but

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# HISTOCHEMICAL LOCALIZATION OF DNA AND RNA IN *CUSCUTA REFLEXA* AND ITS HOST

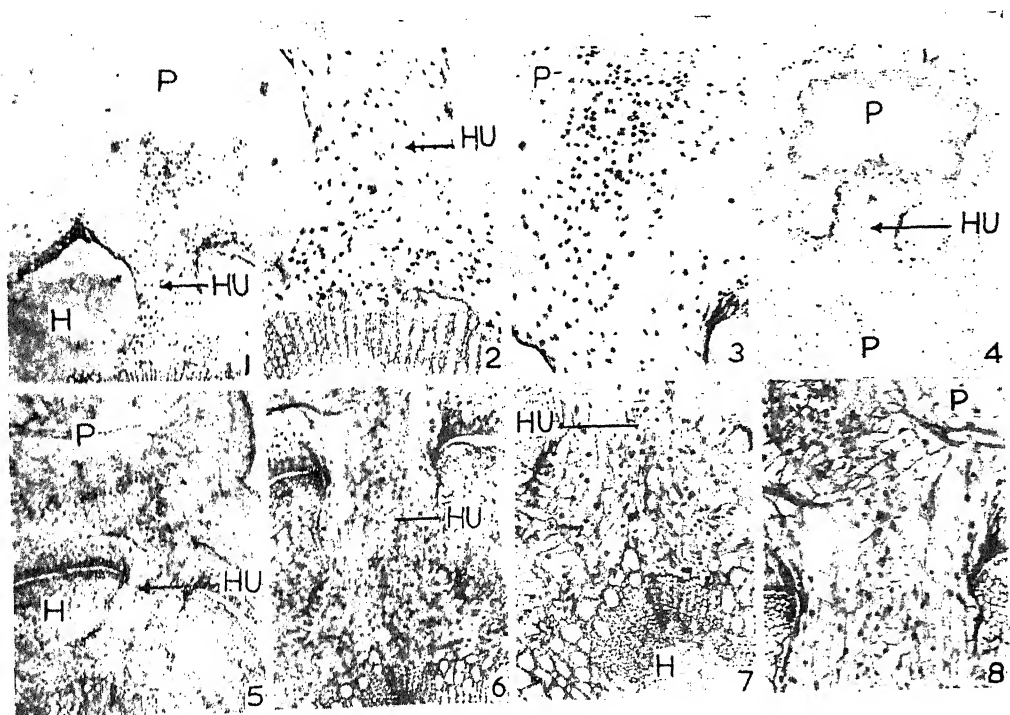
*Cuscuta reflexa* Roxb. is a stem parasite which derives nourishment through haustoria from a variety of host-plants. Qualitative microscopic histochemical studies have been undertaken for *in situ* localization of various metabolites. This communication deals only with the distribution of nucleic acids in *C. reflexa* and its host *Thevetia peruviana* (Pers.) Merr. (Apocynaceae).

Segments of stem with host-parasite union were fixed in F.A.A. for 24 hours. Conventional methods of dehydration and paraffin embedding were followed. Sections cut at 18 microns have been stained with Feulgen method (Gomori)<sup>1</sup> for DNA localization, and Pyronin-Y technique (Tepper and Gilford)<sup>2</sup> for RNA. Adequate control reactions have been conducted.

gate, and invade the host tissue. Longisections of haustorium reveal central vascular tissue consisting of xylem surrounded by phloem, and, parenchymatous cortex. The wedge-shaped haustorium, after entering the cortex of host stem, branches to increase the area of contact with vascular tissue of the host.

The DNA in parasite-stem stains intensely with Feulgen reaction, as compared to the host (Fig. 1). The haustorial region of parasite shows numerous hypertrophied nuclei. These nuclei take dense stain, and indicate a high DNA-content. The hypertrophy of nuclei is maximal in the haustorium. The epidermis of the parasite, in contact with the host, is papillate and has hypertrophied nuclei rich in DNA.

In nature, either various parts of the same parasite-stem, or stems of two separate parasites,



FIGS. 1-4. DNA. 1. T.s. stem of *Cuscuta* along with host, passing through haustorium,  $\times 25$ . 2. Haustorial cells in contact with vasculature of host stem,  $\times 64$ . 3. Cortical parenchyma cells of *Cuscuta* stem adjacent to haustorium,  $\times 64$ . 4. L.s. 'haustorium', between two stems of *Cuscuta*,  $\times 25$ . 5-8. RNA. 5. T.s. stem of *Cuscuta* with host, passing through haustorium,  $\times 25$ . 6. Enlarged view of haustorium,  $\times 45$ . 7. Union of vasculature of haustorium and host,  $\times 64$ . 8. Cortical cells of *Cuscuta* stem adjacent to haustorium,  $\times 64$ .  
(H—host; P—parasite; HU—haustorium).

A haustorium originates from the cortical parenchyma of parasitic stem, as a mass of meristematic cells. These cells undergo repeated divisions elongate, and invade the host tissue. Longisections of haustorium reveal central vascular tissue consisting of xylem surrounded by phloem, and, parenchymatous cortex. The wedge-shaped haustorium, after entering the cortex of host stem, branches to increase the area of contact with vascular tissue of the host.

The DNA in parasite-stem stains intensely with Feulgen reaction, as compared to the host (Fig. 1). The haustorial region of parasite shows numerous hypertrophied nuclei. These nuclei take dense stain, and indicate a high DNA-content. The hypertrophy of nuclei is maximal in the haustorium. The epidermis of the parasite, in contact with the host, is papillate and has hypertrophied nuclei rich in DNA.

In nature, either various parts of the same parasite-stem, or stems of two separate parasites,

is similar to the one described between a true host and a parasite.

RNA concentration, similarly, is much more in the parasite when compared with the host (Fig. 2). Differential distribution of RNA is observed within the parasite; being abundant in the haustorial cells and cortical parenchyma adjacent to the haustorium. The papillate epidermal cells of the parasite-stem are also rich in RNA.

This distribution pattern of DNA and RNA in the haustorium suggests its active metabolic state, and probable role in the conduction of food materials from the host to the parasite.

We are grateful to Professor B. M. Johri for encouragement. One of us (VMR) acknowledges the award of a Junior Research Fellowship by the Council of Scientific and Industrial Research, New Delhi.

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#### INHIBITION OF $GA_3$ INDUCED EXTENSION GROWTH AND MALE FLOWER FORMATION IN FEMALE PLANTS OF *CANNABIS SATIVA* BY CYCLOHEXIMIDE

THE various plant processes affected by gibberellin treatment have been enumerated by Paleg<sup>1</sup> and the physiological role of gibberellins has been recently reviewed by Jones<sup>2</sup>. Responses such as production of hydrolases and synthesis of endoplasmic reticulum in barley aleurone cells have been shown to be inhibited by cycloheximide, puromycin, 6-methylpurine, chloramphenicol and actinomycin-D<sup>3-5</sup>. It was of interest to verify whether responses like extension growth and production of male flowers in female plants caused by gibberellin treatment in *Cannabis sativa*<sup>6-7</sup>, were also affected by metabolic inhibitors. The present communication describes our findings on the effect of interaction between  $GA_3$  and a protein synthesis inhibitor, cycloheximide.

Seedlings of *C. sativa* were raised and only female plants were selected for study. Ten plants were used for each treatment. Gibberellic acid ( $GA_3$ ) and cycloheximide (CH) were applied in cotton wicks to the shoot apices separately and in combination; control plants received only distilled water. Shoot length, number of nodes bearing male flowers and the total number of male flowers in each treated plant were recorded.

Plants treated with CH alone at 1–50  $\mu$ g/plant showed reduction in extension growth without affecting flower sex (Table I). Plants receiving 25, 50 and 100  $\mu$ g of  $GA_3$  showed marked enhancement in extension growth and bore male flowers in the newly formed nodes. At 75 and 100  $\mu$ g/plant CH caused injury and drying up of the shoot tips.

Application of 25  $\mu$ g of  $GA_3$  and CH (10 and 25  $\mu$ g/plant) together caused marked reduction in the number of nodes producing male flowers and a slight inhibition in extension growth. However, when CH at 50  $\mu$ g was applied together with  $GA_3$  at 25  $\mu$ g it completely inhibited the production of male flowers and substantially decreased stem elongation (Table I).

TABLE I  
*Effects of  $GA_3$  and cycloheximide and their interaction on extension growth and male flower formation in female plants of *C. sativa**

Treatment*	Extension growth† (cm)	Number of nodes/plant with ♂ flowers	Number of ♂ flowers/plant
Control	88	0	0
CH 1	86	0	0
CH 10	80	0	0
CH 25	67	0	0
CH 50	49	0	0
CH 75	Shoot apex injured		0
CH 100	"		0
$GA_3$ 25	168	2.2	28.3
$GA_3$ 25+CH 1	165	2.1	26.0
$GA_3$ 25+CH 10	160	1.7	19.6
$GA_3$ 25+CH 25	131	0.6	8.0
$GA_3$ 25+CH 50	110	0.0	0.0
$GA_3$ 25+CH 75	Shoot apex injured		25.0
$GA_3$ 25+CH 100	"		29.0
$GA_3$ 50	180	3.8	45.3
$GA_3$ 50+CH 1	176.5	3.7	44.6
$GA_3$ 50+CH 10	172	3.4	40.0
$GA_3$ 50+CH 25	163	2.7	35.0
$GA_3$ 50+CH 50	140	1.3	13.0
$GA_3$ 50+CH 75	Shoot apex injured		47.0
$GA_3$ 50+CH 100	"		43.0
$GA_3$ 100	210	6.7	103.0
$GA_3$ 100+CH 1	208	6.6	101.0
$GA_3$ 100+CH 10	201	6.3	94.0
$GA_3$ 100+CH 25	188	5.2	83.0
$GA_3$ 100+CH 50	159	4.1	56.0
$GA_3$ 100+CH 75	Shoot apex injured		107
$GA_3$ 100+CH 100	"		104.5

\* Total amount of chemicals applied in  $\mu$ g/plant.  
† Growth period=8 weeks.

In interaction experiments in which the amount of  $GA_3$  was raised to 50 and 100  $\mu$ g/plant, and that of CH to 25 and 50  $\mu$ g, the number of nodes with male flowers was not totally inhibited although

extension growth was somewhat reduced. At no concentration tried was CH able to completely inhibit the  $GA_3$  response. However, when  $GA_3$  is applied at 50 and 100  $\mu g$  in combination with CH at 75 and 100  $\mu g$ /plant, the shoot apex of the treated plants dried up and the laterals which were formed immediately below the apex produced male flowers. It is a general finding that application of  $GA_3$  alone to the shoot tip invariably produces male flowers on the main shoot in the nodes that differentiate after treatment<sup>6,7</sup>.

It may be inferred that CH at 75 and 100  $\mu g$ /plant proved highly toxic to the tissues of the shoot apex and was also immobile. Death of the apex inhibited the growth of the laterals.  $GA_3$  being a mobile moved to the lateral shoots and stimulated male flower formation at the nodes.

Although the main apex was injured in inter-nodal experiments containing CH at 75 and 100  $\mu g$ /plant, the total number of male flowers formed was not significantly different from those treatments in which  $GA_3$  alone was applied.

Several responses caused by gibberellin treatment are shown to be mediated through synthesis of gibberellins. If it is presumed that for the induction of male flowers in female plants of *Cannabis* a new enzyme must be synthesized through gibberellin

treatment, the inhibition of such a response by cycloheximide may be presumed to be brought about by the inhibition of the specific enzyme.

We extend our thanks to Dr. S. R. V. Rao, Department of Zoology, University of Delhi, for providing cycloheximide. One of us (V. S. J.) acknowledges the award of a Senior Research Fellowship by the Council of Scientific and Industrial Research, New Delhi.

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## SHORT SCIENTIFIC NOTES

*Galactosomum lacteum* (Jagerskiold, 1896) Looss, 1899 (Trematoda: Heterophyidae) from a Domestic Cat (*Felis catus* Linne.) in Madras, India

In India, Anantaraman<sup>1</sup> recorded four species of *Galactosomum*, three of them as adults in the sea and one, as a juvenile in crabs besides certain reports of *Galactosomum* sp. in the marine gastropod<sup>1</sup> and fishes<sup>2</sup>. But *G. lacteum* has never been encountered in India nor has ever been recovered from the domestic cat. Hence the recovery of the parasite from a domestic cat (*Felis catus* Linne.) in Madras is reported herein for the first time besides furnishing additional details on its morphological features.

An adult female cat was autopsied on 17-8-1968. Examination of endoparasites and among the contents of its small intestine were found three adult and four juvenile trematodes which were identified as *Galactosomum lacteum*.

In the adult parasite the prepharynx is faintly observed while the oesophagus is indistinct. The gonotyl is retractile and is covered with minute

spines arranged in two diagonally placed groups connected by rows of spines in the form of an isthmus. The seminal vesicle is well developed and muscular, broad posteriorly and narrow anteriorly and opens into the genital atrium. Prudhoe<sup>3</sup> in his detailed account on the morphology of *G. lacteum* has not mentioned the very small spines coating the gonotyl. The spines could not be observed in the juveniles.

The fact that the parasite specimens were recovered from a cat which was autopsied about 12 hours after its capture, fairly rules out the possibility of the cat's recent access to shore bird (as prey) harbouring them. It is quite possible that the cat had acquired the infection on ingestion of infected intermediate host, viz., the fish, in which the metacercariae are known to occur. It should be noted that three of the seven specimens, obtained from the cat, were adults and none occurred in the encysted stage. The present finding, therefore, indicates that *G. lacteum* is capable of establishing itself as adult in the domestic cat, and its success

in using the cat as a final host remains to be confirmed by experimental infection studies.

This work formed part of the dissertation for the M.V.Sc. degree of the University of Madras submitted by the first author and grateful acknowledgement is made of the facilities provided by the Dean, Madras Veterinary College, for the same.

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#### Post-Infection Changes in Sugar Contents of Banana Fruits

Ripe fruits of 'Alpan' variety of Banana (*Musa paradisiaca* L.) were inoculated with *Alternaria tenuis* Auct and *Helminthosporium speciferum* (Bain) Nicot and were incubated at  $25^{\circ} \pm 1^{\circ}$  C. Tissue adjacent to the inoculated region was analysed on alternate days upto 10 days. For the detection of sugars, thin layer chromatographic technique adopted by Stahl (1965) was employed. *n*-Butanol-acetone-water (4 : 5 : 1) was used as the solvent and spots were developed by spraying aniline phthalate solution (0.93 gm aniline and 1.66 gm of phthalic acid dissolved in 100 ml water saturated with *n*-butanol). The intensities of the bands were compared visually and according to their concentration, they were graded in five categories, i.e., 5+, 4+, 3+, ..., 1+. The results are presented in Table I.

Soluble sugars sucrose, glucose and fructose were present in the fruits. In healthy fruits, the concentrations of sucrose and glucose were higher than those of fructose. A well-marked difference was expressed by *A. tenuis* and *H. speciferum* in their comparative rates of utilization. With the increase of the incubation period the concentration of sucrose, glucose and fructose decreased. *A. tenuis* consumed fructose earlier than *H. speciferum*. It is interesting that both the fungi hydrolysed sucrose rather slowly and even after 10 days, some fructose and glucose persisted.

TABLE I  
Presence of various sugars in banana fruits during advancement of rot caused by *Alternaria tenuis* and *Helminthosporium speciferum*

Days of incubation	Sucrose	Glucose	Fructose
Healthy			
0	4+	4+	2+
2	4+	4+	3+
4	3+	3+	4+
6	4+	2+	4+
8	4+	3+	3+
10	4+	4+	3+
<i>A. tenuis</i>			
0	4+	4+	2+
2	3+	3+	3+
4	3+	3+	3+
6	3+	3+	2+
8	2+	2+	1+
10	2+	2+	1+
<i>H. speciferum</i>			
0	4+	4+	2+
2	0+	2+	1+
4	3+	3+	3+
6	3+	4+	3+
8	3+	2+	2+
10	2+	2+	2+

Several studies<sup>1-3</sup> have shown the formation of transient oligosaccharides by pathogenic fungi on sucrose solution. Formation of oligosaccharides in mango and other fruits under pathogenesis is also known<sup>5-6</sup>. We were, however, unable to spot transient oligosaccharides in banana fruits under pathogenesis which is obviously due to slow rate of conversion of sucrose and simultaneous utilization of the monosaccharide component.

The author is grateful to Professor K. S. Bilgrami, for his guidance and necessary laboratory facilities and to Dr. R. N. Verma and Sri A. K. Roy for various kinds of help.

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**Occurrence of the Hyperparasite *Cerebella* on Ergot of Bajra [*Pennisetum typhoides* (Burm F.) Stapf and C. E. Hubb.]**

During the course of routine survey in the Kharif season for phytopathogenic fungi in Kaira District of Gujarat State unusual infection spots were noticed overgrowing on the ergot affected spikelets of Bajra (*Pennisetum typhoides*). The infection was characterized by raised sticky masses of fungal colonies turning the affected spikelets to dark thick compact pustules. The infection was restricted to ergot affected spikelets showing its hyperparasitic nature resulting in non-development of the ergot bodies. Microscopic examination of the fungal pustules revealed them to be in the nature of sporodochia of a species of *Cerebella*. A search through literature revealed no report of any species of *Cerebella* on Bajra. So far although Ajrekar<sup>1</sup> has reported *Cerebella sorghi vulgaris*, L. S. Subr. as a hyperparasite on sugary disease (*Sphacelia orghi*) of Sorghum.

**Diagnosis and Identification of the Fungus:**

Sporodochia convoluted, dark, compact, conidiospores short, often branched, pale brown, smooth, measure 3-6  $\mu$  in length. Conidia terminal, multi-cellular (avg. 4 to 5), variable in shape, globular, muriform with cross to oblique septa, constricted at septa, smooth walled, with basal cell, brown to dark-brown, measure 7.40-25.60  $\times$  7.40-22  $\mu$  (avg. 6.60  $\times$  13.40  $\mu$ ). On the basis of these characters and dimensions, the fungus was identified as *Cerebella andropogonis* Ces<sup>2</sup>.

This constitutes the first known report of *Cerebella andropogonis* Ces. on ergot [*Claviceps microcephala* Waller] Tul.] of Bajra.

The material has been deposited at the Ajrekar Mycological Herbarium of M.A.C.S., Poona, under No. AMH 2201.

Grateful thanks are offered to Prof. M. N. Kamat for his keen interest, to the Director of the Institute for laboratory facilities and to Dr. V. G. Rao, for his valuable help during the course of investigation.

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**Insect Antifeedants Against Snail *Opeas gracile* (Hutton)**

Insect antifeedants are chemicals preventing insects from feeding on treated foliage, without killing or even repelling them. They have been found successful against insect pests by many workers in India and abroad<sup>1,2</sup>. The present note reports results on the effect of three insect antifeedants on a snail, *Opeas gracile* H. attacking vegetable crops in Kerala.

Pre-weighed pea leaves were sprayed with fentin acetate, fentin chloride and AC-24055 at 0.1 and 0.2% concentrations using an atomiser. Leaves sprayed with distilled water were kept as control. After air-drying, the leaves were transferred to glass chimneys over petri-dishes and ten snails of equal age were liberated inside for feeding *ad libitum*. Leaves were also kept as check to assess the natural reduction in weight during the experimental period of 48 hours. The leaves were again weighed after the experiment.

All the three compounds successfully inhibited the feeding activity of the snails. The triazene compound AC-24055 ranked first followed by fentin chloride and fentin acetate. The corrected percentages of leaf protected by weight were 60.8, 67.9, 65.4, 74.3, 95.7 and 100.0 in 0.1 and 0.2% fentin acetate, fentin chloride and AC-24055 compound respectively.

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**The Pachytene Chromosome of Jute (*Corchorus olitorius* L.)**

Dr. R. M. Datta, Agricultural Department, Calcutta University, writes that the information about "The pachytene chromosomes of Jute (*Corchorus olitorius* L.)" published by P. Paria and S. L. Basak (*Current Science*, 1973, 42, p. 832) has already been reported by him in *Nucleus*, 1968, p. 43.

## REVIEWS AND NOTICES OF BOOKS

**Fundamentals of High Altitude Biology.** By M. S. Mani. (Oxford and IBH Publishing Co., New Delhi and Calcutta), 1974. Pp. 196. Price Rs. 42-00.

High altitude situations all over the world offer a unique, often hostile, environment for the existence, functioning and survival of biological organisms. Characterized by drastic reduction in temperature, atmospheric pressure and precipitation, high altitudes on earth yet harbour a variety of organisms, plants and animals, which show great adaptability to these conditions. The book under review presents an account of the physical characteristics of high altitudes on this planet, enumerates the plants and animals that inhabit them and goes on to relate the biology of these organisms with the environment in which they live.

Dr. Mani is an Entomologist who has made High altitude biology of insects his life study. Originally interested in the insects of the Himalayas, he has had, more recently, opportunities of studying the high altitude insect life of South Asia,—the Alai-Pamirs, The Tien Shan and the Caucasus. He is an author of several books on High Altitude Entomology and is one of the recognized experts on the subject.

The present book deals with several aspects of Entomology that formed the subject-matter of his earlier treatises, but differs in that he devotes much space, in fact nearly half of the text, to a consideration of Man at High altitude. About this, more later.

Indian is uniquely possessed of situations of high altitude. The entire Himalayan range offers unequalled high altitude situations, and much of it, especially in the West, forms part of the author's material. High altitude, which for practical considerations, could be regarded as anything 3000 m above sea level, by itself is not an important ecological factor. What is important is the reduction in atmospheric pressure, and it is this that brings about other chain effects,—in temperature, precipitation, winds, aridity, treelessness. Of these, the absence of trees is perhaps the most striking. Trees as influencers of climate, soil conditions, temperature, etc., are well known and the total absence of trees has profound effects on high altitude ecology.

No better example of the close relationship between the several biotic and non-biotic factors

that make up the environment can be found than in high altitudes. The low temperatures and pronounced aridity have resulted in reduction of insects, which in its turn has reduced insect pollination to a minimum. The flowers in high altitude plants lack scent and other devices to attract insects. On the other hand, wind pollination is far more pronounced. The insects that do inhabit high altitudes are characterized by aptery, melanism and small body size. They are largely diurnal and tend to crowd in restricted areas, feeding on debris and carrion. However, low-land insects occur quite often in these areas and are accountable under two heads: (1) Several low-land forms get carried by winds and currents of air and are often found in inaccessible places, inactive and frozen. These are the "aeolian derelicts" of Mani. They evidently are of little significance except that they provide food for genuine h.a. insects. (2) On the other hand, several species of low-land insects in large numbers appear attracted to mountain summits and deliberately get there. This "summit-seeking" remains an enigma and in spite of several attempts to explain the phenomenon, where often several thousand individuals appear compulsively to seek high altitudes, no satisfactory solution is available. Mani once found over 2 million *Coccinella* and *Hippodamia* at an elevation of 4,260 m in the Himalayas.

Mani devotes nearly half of his text to a discussion of Man in high altitudes. Over 10 million people live permanently at these altitudes (3,600 m–5,300 m). Most of them in the Peruvian Andes. The Himalayas are of little significance in this regard, also because little work has been done in this country. Admittedly Mani relies on the information provided by h.a. studies in the Andes and this information is extensive. Man's advent in this environment is relatively recent. Investigations show that Man has made high altitude living only during the past 10,000 years. It is still a hostile environment to him and it is too soon to expect any harmony in the adaptation of his structure and physiology to his surroundings.

The book is a useful contribution to the ecology of a special environment on this planet. Its unique feature is an extensive bibliography with nearly 450 citations covering 35 pages out of a total of less than 200. One however is discouraged at the high cost of the book.

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